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(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF IMMUNE RELATED DISEASES

MAALASSLI RQKREVREPGGSRPVSAQRVCPRGTKSLCQKQLLILLSKVRLCGGRPARPDRGPEPQLKGIVTK  
LFCRQGFYLOANPDGSIQGTPEDTSSFTHEMLIPVGLRVVTIQSAKLGHYAMNAEGLLYSSPHETAECRFKEC  
VFENYYVLYASALYRQRRSGRAWYLGLDKEGQVMKGNRVKTKAAAHFLPKLLEVAMYQEPSLHVSVEASPPSP  
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(57) Abstract: The present invention relates to a composition containing novel proteins and methods for the diagnosis and treatment of immune related diseases.



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## COMPOSITIONS AND METHODS FOR THE TREATMENT OF IMMUNE RELATED DISEASES

Field of the Invention

5       The present invention relates to compositions and methods for the diagnosis and treatment of immune related diseases.

Background of the Invention

10       Immune related and inflammatory diseases are the manifestation or consequence of fairly complex, often multiple interconnected biological pathways which in normal physiology are critical to respond to insult or injury, initiate repair from insult or injury, and mount innate and acquired defense against foreign organisms. Disease or pathology occurs when these normal physiological pathways cause additional insult or injury either as directly related to the intensity of the response, as a consequence of abnormal regulation or excessive stimulation, as a reaction to self, or as a combination of these.

15       Though the genesis of these diseases often involves multistep pathways and often multiple different biological systems/pathways, intervention at critical points in one or more of these pathways can have an ameliorative or therapeutic effect. Therapeutic intervention can occur by either antagonism of a detrimental process/pathway or stimulation of a beneficial process/pathway.

20       Many immune related diseases are known and have been extensively studied. Such diseases include immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, etc.

25       T lymphocytes (T cells) are an important component of a mammalian immune response. T cells recognize antigens which are associated with a self-molecule encoded by genes within the major histocompatibility complex (MHC). The antigen may be displayed together with MHC molecules on the surface of antigen presenting cells, virus infected cells, cancer cells, grafts, etc. The T cell system eliminates these altered cells which pose a health threat to the host mammal. T cells include helper T cells and cytotoxic T cells. Helper T cells proliferate extensively following recognition of an antigen-MHC complex on an antigen presenting cell. Helper T cells also secrete a variety of cytokines, i.e., lymphokines, which play a central role in the activation of B cells, cytotoxic T cells and a variety of other cells which participate in the immune response.

30       A central event in both humoral and cell mediated immune responses is the activation and clonal expansion of helper T cells. Helper T cell activation is initiated by the interaction of the T cell receptor (TCR) - CD3 complex with an antigen-MHC on the surface of an antigen presenting cell. This interaction mediates a cascade of biochemical events that induce the resting helper T cell to enter a cell cycle (the G0 to G1 transition) and results in the expression of a high affinity receptor for IL-2 and sometimes IL-4. The activated T cell progresses through the cycle proliferating and differentiating into memory cells or effector cells.

35       In addition to the signals mediated through the TCR, activation of T cells involves additional costimulation induced by cytokines released by the antigen presenting cell or through interactions with membrane bound molecules on the antigen presenting cell and the T cell. The cytokines IL-1 and IL-6 have been shown to provide a costimulatory signal. Also, the interaction between the B7 molecule expressed on the surface of an antigen presenting cell and CD28 and CTLA-4 molecules expressed on the T cell surface effect T cell activation. Activated T cells express an increased number of cellular adhesion molecules, such as ICAM-1,

40



integrins, VLA-4, LFA-1, CD56. etc.

T-cell proliferation in a mixed lymphocyte culture or mixed lymphocyte reaction (MLR) is an established indication of the ability of a compound to stimulate the immune system. In many immune responses, inflammatory cells infiltrate the site of injury or infection. The migrating cells may be neutrophilic, eosinophilic, monocytic or lymphocytic as can be determined by histologic examination of the affected tissues. *Current Protocols in Immunology*, ed. John E. Coligan, 1994, John Wiley & Sons, Inc. T-cell activity is also positively affected by stimulation with anti-CD3 and anti-CD28 antibodies. Thus, the ability of a compound to inhibit the costimulation or alternatively replace anti-CD28 is indicative of the inhibitory or stimulatory effect, respectively, on the immune system.

Immune related diseases can be treated by suppressing the immune response. Using neutralizing antibodies that inhibit molecules having immune stimulatory activity would be beneficial in the treatment of immune-mediated and inflammatory diseases. Molecules which inhibit the immune response can be utilized (proteins directly or via the use of antibody agonists) to inhibit the immune response and thus ameliorate immune related disease.

#### Summary of the Invention

##### A. Embodiments

The present invention concerns compositions and methods for the diagnosis and treatment of immune related disease in mammals, including humans. The present invention is based on the identification of proteins (including agonist and antagonist antibodies) which either stimulate or inhibit the immune response in mammals. Immune related diseases can be treated by suppressing or enhancing the immune response. Molecules that enhance the immune response stimulate or potentiate the immune response to an antigen. Molecules which stimulate the immune response can be used therapeutically where enhancement of the immune response would be beneficial. Alternatively, molecules that suppress the immune response attenuate or reduce the immune response to an antigen (e.g., neutralizing antibodies) can be used therapeutically where attenuation of the immune response would be beneficial (e.g., inflammation). Accordingly, the PRO polypeptides, agonists and antagonists thereof are also useful to prepare medicines and medicaments for the treatment of immune-related and inflammatory diseases. In a specific aspect, such medicines and medicaments comprise admixing a therapeutically effective amount of a PRO polypeptide, agonists or antagonist thereof with a pharmaceutically acceptable carrier. Preferably, the admixture is sterile.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprises contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native sequence PRO polypeptide. In a specific aspect, the PRO agonists or antagonist is an anti-PRO antibody.

In another embodiment, the invention concerns a composition of matter containing PRO polypeptide or an agonist or antagonist antibody which binds the polypeptide in admixture with a carrier or excipient. In one aspect, the composition contains a therapeutically effective amount of the peptide or antibody. In another aspect, when the composition contains an immune stimulating molecule, the composition is useful for: (a) increasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) stimulating or enhancing an immune response in a mammal in need thereof, (c) increasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen, (d) stimulating the activity of T-lymphocytes or (e)

increasing the vascular permeability. In a further aspect, when the composition contains an immune inhibiting molecule, the composition is useful for: (a) decreasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) inhibiting or reducing an immune response in a mammal in need thereof, (c) decreasing the activity of T-lymphocytes or (d) decreasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen. In another aspect, the composition contains a further active ingredient, which may, for example, be a further antibody or a cytotoxic or chemotherapeutic agent. Preferably, the composition is sterile.

In another embodiment, the invention concerns a method of treating an immune related disorder in a mammal in need thereof, comprising administering to the mammal an effective amount of a PRO polypeptide, an agonist thereof, or an antagonist thereto. In a preferred aspect, the immune related disorder is selected from the group consisting of: systemic lupus erythematosus, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis, idiopathic inflammatory myopathies, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious, autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft-versus-host-disease.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody. In one aspect, the present invention concerns an isolated antibody which binds a PRO polypeptide. In another aspect, the antibody mimics the activity of a PRO polypeptide (an agonist antibody) or conversely the antibody inhibits or neutralizes the activity of a PRO polypeptide (an antagonist antibody). In another aspect, the antibody is a monoclonal antibody, which preferably has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a monoclonal antibody, a single-chain antibody, or an anti-idiotypic antibody.

In yet another embodiment, the present invention provides a composition comprising an anti-PRO antibody in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the antibody. Preferably, the composition is sterile. The composition may be administered in the form of a liquid pharmaceutical formulation, which may be preserved to achieve extended storage stability. Alternatively, the antibody is a monoclonal antibody, an antibody fragment, a humanized antibody, or a single-chain antibody.

In a further embodiment, the invention concerns an article of manufacture, comprising:

- (a) a composition of matter comprising a PRO polypeptide or agonist or antagonist thereof;

(b) a container containing said composition; and  
(c) an instruction affixed to said container, or a package insert included in said container referring to the use of said PRO polypeptide or agonist or antagonist thereof in the treatment of an immune related disease. The composition may comprise a therapeutically effective amount of the PRO polypeptide or the agonist or antagonist thereof.

In yet another embodiment, the present invention concerns a method of diagnosing an immune related disease in a mammal, comprising detecting the level of expression of a gene encoding a PRO polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control sample indicates the presence of immune related disease in the mammal from which the test tissue cells were obtained.

In another embodiment, the present invention concerns a method of diagnosing an immune disease in a mammal, comprising (a) contacting an anti-PRO antibody with a test sample of tissue cells obtained from the mammal; and (b) detecting the formation of a complex between the antibody and the respective PRO polypeptide, respectively, in the test sample; wherein the formation of said complex is indicative of the presence or absence of said disease. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger quantity of complexes formed in the test sample indicates the presence or absence of an immune disease in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. The test sample is usually obtained from an individual suspected of having a deficiency or abnormality of the immune system.

In another embodiment, the invention provides a method for determining the presence of a PRO polypeptide in a sample comprising exposing a test samples of cells suspected of containing the PRO polypeptide to an anti-PRO antibody and determining the binding of said antibody to a component of said cell sample. In a specific aspect, the sample comprises a cell suspected of containing the PRO polypeptide and the antibody binds to the cell. The antibody is preferably detectably labeled and/or bound to a solid support.

In another embodiment, the present invention concerns an immune-related disease diagnostic kit, comprising an anti-PRO antibody and a carrier in suitable packaging. The kit preferably contains instructions for using the antibody to detect the presence of the PRO polypeptide. Preferably the carrier is pharmaceutically acceptable.

In another embodiment, the present invention concerns a diagnostic kit, containing an anti-PRO in suitable packaging. The kit preferably contains instructions for using the antibody to detect the PRO polypeptide.

In another embodiment, the invention provides a method of diagnosing an immune-related disease in a mammal which comprises detecting the presence or absence of a PRO polypeptide in a test sample of tissue cells obtained from said mammal, wherein the presence or absence of a PRO polypeptide in said test sample is indicative of the presence of an immune-related disease in said mammal.

In another embodiment, the present invention concerns a method for identifying an agonist of a PRO polypeptide comprising:

(a) contacting cells and a test compound to be screened under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective agonist, herein the induction of said cellular response is indicative of said test compound being an effective agonist.

In another embodiment, the present invention provides a method for identifying an agonist of a PRO polypeptide comprising:

(a) contacting cells and a test compound to be screened under conditions suitable for the stimulation of cell proliferation by a PRO polypeptide; and

(b) measuring the proliferation of said cells to determine if the test compound is an effective agonist, wherein the stimulation of cell proliferation is indicative of said test compound being an effective agonist.

In another embodiment, the invention concerns a method for identifying a compound capable of inhibiting the expression and/or activity of a PRO polypeptide by contacting a candidate compound with a PRO polypeptide under conditions and for a time sufficient to allow these two components to interact and determining whether the activity of the PRO polypeptide is inhibited. In a specific aspect, either the candidate compound or the PRO polypeptide is immobilized on a solid support. In another aspect, the non-immobilized component carries a detectable label. In a preferred aspect, this method comprises the steps of:

(a) contacting cells and a test compound to be screened in the presence of a PRO polypeptide under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective antagonist. In another preferred aspect, the method comprises the step of:

(a) contacting cells and a test compound to be screened in the presence of a PRO polypeptide under conditions suitable for the stimulation of cell proliferation by a PRO polypeptide under conditions suitable for the stimulation of cell proliferation by a PRO polypeptide; and

(b) measuring the proliferation of the cells to determine if the test compound is an effective antagonist.

In another embodiment, the invention provides a method for identifying a compound that inhibits the expression of a PRO polypeptide in cells that normally express the polypeptide, wherein the method comprises contacting the cells with a test compound and determining whether the expression of the PRO polypeptide is inhibited. In a preferred aspect, this method comprises the steps of:

(a) contacting cells and a test compound to be screened under conditions suitable for allowing expression of the PRO polypeptide; and

(b) determining the inhibition of expression of said polypeptide.

In yet another embodiment, the present invention concerns a method for treating an immune-related disorder in a mammal that suffers therefrom comprising administering to the mammal a nucleic acid molecule that codes for either (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide or (c) an antagonist of a PRO polypeptide, wherein said agonist or antagonist may be an anti-PRO antibody. In a preferred embodiment, the mammal is human. In another preferred embodiment, the nucleic acid is administered via *ex vivo* gene therapy. In a further preferred embodiment, the nucleic acid is comprised within a vector, more preferably an adenoviral, adeno-associated viral, lentiviral or retroviral vector.

In yet another aspect, the invention provides a recombinant retroviral particle comprising a retroviral

vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide of a PRO polypeptide, or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein the retroviral vector is in association with retroviral structural proteins. Preferably, the signal sequence is from a mammal, such as from a native PRO polypeptide.

5 In a still further embodiment, the invention concerns an *ex vivo* producer cell comprising a nucleic acid construct that expresses retroviral structural proteins and also comprises a retroviral vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide or a PRO polypeptide or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein said producer cell packages the retroviral vector in association with the structural proteins  
10 to produce recombinant retroviral particles.

In a still further embodiment, the invention provides a method for increasing the infiltration of inflammatory cells from the vasculature into a tissue of a mammal comprising administering a therapeutically effective amount of (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the infiltration of inflammatory cells from the vasculature in the mammal is increased.

15 In a still further embodiment, the invention provides a method for decreasing the infiltration of inflammatory cells from the vasculature into a tissue of a mammal comprising administering a therapeutically effective amount of (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the infiltration of inflammatory cells from the vasculature in the mammal is decreased.

In a still further embodiment, the invention provides for a method of increasing the activity of T-lymphocytes in a mammal comprising administering a therapeutically effective amount of (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the activity of T-lymphocytes in the mammal is increased.

20 In a still further embodiment, the invention provides for a method of decreasing the activity of T-lymphocytes in a mammal comprising administering a therapeutically effective amount of (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the activity of T-lymphocytes in the mammal is decreased.

In a still further embodiment, the invention provides for a method of increasing the proliferation of T-lymphocytes in a mammal comprising administering a therapeutically effective amount of (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the proliferation of T-lymphocytes in the mammal is increased.

30 In a still further embodiment, the invention provides for a method of decreasing the proliferation of T-lymphocytes in a mammal comprising administering a therapeutically effective amount of (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the proliferation of T-lymphocytes in the mammal is decreased.

35 In a still further embodiment, the invention provides for a method of affecting the proliferation of T-cells comprising contacting PBMC cells with an effective amount of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1065, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940, PRO6006 polypeptide and  
40 measuring the change in proliferation from control levels.

In a still further embodiment, the invention provides for of stimulating the activity of T-cells comprising contacting CD4+ cells or PBMC cells with an effective amount of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940, PRO6006 polypeptide in combination with an effective amount of anti-CD3 antibody and measuring the change in activity from control levels.

In a still further embodiment, the invention provides for a method of inhibiting the activity of T-cells comprising contacting CD4+ cells which have been previously stimulated by treatment with anti-CD3 and anti-CD28 antibodies, with an effective amount of PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940, PRO6006 polypeptide and measuring the change in activity from control levels.

In a still further embodiment, the invention provides for a method of stimulating the proliferation of T-lymphocytes in a mammal comprising administering a therapeutically effective amount of a PRO861, PRO788, PRO1159, PRO1646, PRO1475, PRO1917, PRO9940, PRO5723 or PRO6006 polypeptide, wherein the proliferation of T-lymphocytes in the mammal is stimulated.

In a still further embodiment, the invention provides for a method of decreasing the proliferation of T-lymphocytes in a mammal comprising administering a therapeutically effective amount of a PRO184, PRO306, PRO779, PRO1271, PRO1375 or PRO1474 polypeptide, wherein the proliferation of T-lymphocytes is decreased.

In a still further embodiment, the invention provides for a method of stimulating the activity of T-lymphocytes comprising administering a therapeutically effective amount of a PRO245, PRO266, PRO306, PRO333, PRO356, PRO364, PRO381, PRO526, PRO719, PRO769, PRO826, PRO1031, PRO1069, PRO1343, PRO1375 or PRO1418 polypeptide, wherein the activity of T-lymphocytes is increased.

In a still further embodiment, the invention provides for a method of decreasing the activity of T-lymphocytes comprising administering a therapeutically effective amount of a PRO184, PRO212, PRO306, PRO333, PRO364, PRO381, PRO982, PRO1068, PRO1157, PRO1343, PRO4302 or PRO4405 polypeptide, wherein the activity of T-lymphocytes is decreased.

#### B. Additional Embodiments

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the herein described polypeptides. Host cell comprising any such vector are also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the herein described polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the herein described polypeptides fused to a heterologous polypeptide or amino acid sequence. Example of such chimeric

molecules comprise any of the herein described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized  
5 antibody, antibody fragment or single-chain antibody.

In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences or as antisense probes, wherein those probes may be derived from any of the above or below described nucleotide sequences.

In other embodiments, the invention provides an isolated nucleic acid molecule comprising a  
10 nucleotide sequence that encodes a PRO polypeptide.

In one aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least  
15 about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule encoding a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed  
20 herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein; or (b) the complement of the DNA molecule of (a).

In other aspects, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least  
30 about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule comprising the coding  
40

sequence of a full-length PRO polypeptide cDNA as disclosed herein, the coding sequence of a PRO polypeptide lacking the signal peptide as disclosed herein, the coding sequence of an extracellular domain of a transmembrane PRO polypeptide, with or without the signal peptide, as disclosed herein or the coding sequence of any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule that encodes the same mature polypeptide encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein, or (b) the complement of the DNA molecule of (a).

Another aspect the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated, or is complementary to such encoding nucleotide sequence, wherein the transmembrane domain(s) of such polypeptide are disclosed herein. Therefore, soluble extracellular domains of the herein described PRO polypeptides are contemplated.

Another embodiment is directed to fragments of a PRO polypeptide coding sequence, or the complement thereof, that may find use as, for example, hybridization probes, for encoding fragments of a PRO polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO antibody or as antisense oligonucleotide probes. Such nucleic acid fragments are usually at least about 20 nucleotides in length, alternatively at least about 30 nucleotides in length, alternatively at least about 40 nucleotides in length, alternatively at least about 50 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 70 nucleotides in length, alternatively at least about 80 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 100 nucleotides in length, alternatively at least about 110 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 130 nucleotides in length, alternatively at least about 140 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 160 nucleotides in length, alternatively at least about 170 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 190 nucleotides in length, alternatively at least about 200 nucleotides in length, alternatively at least about 250 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 350 nucleotides in length, alternatively at least about 400 nucleotides in length,



alternatively at least about 450 nucleotides in length, alternatively at least about 500 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 700 nucleotides in length, alternatively at least about 800 nucleotides in length, alternatively at least about 900 nucleotides in length and alternatively at least about 1000 nucleotides in length, wherein in this context the term "about" means the  
5 referenced nucleotide sequence length plus or minus 10% of that referenced length. It is noted that novel fragments of a PRO polypeptide-encoding nucleotide sequence may be determined in a routine manner by aligning the PRO polypeptide-encoding nucleotide sequence with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which PRO polypeptide-encoding nucleotide sequence fragment(s) are novel. All of such PRO polypeptide-encoding nucleotide sequences are  
10 contemplated herein. Also contemplated are the PRO polypeptide fragments encoded by these nucleotide molecule fragments, preferably those PRO polypeptide fragments that comprise a binding site for an anti-PRO antibody.

In another embodiment, the invention provides isolated PRO polypeptide encoded by any of the isolated nucleic acid sequences hereinabove identified.

15 In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity,  
20 alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid  
25 sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically  
30 defined fragment of the full-length amino acid sequence as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at  
35 least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity,  
40 alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid

sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to an amino acid sequence encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein.

5 In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence scoring at least about 80% positives, alternatively at least about 81% positives, alternatively at least about 82% positives, alternatively at least about 83% positives, alternatively at least about 84% positives, alternatively at least about 85% positives, alternatively at least about 86% positives, alternatively at least about 87% positives, alternatively at least about 88% positives, alternatively at least about 89% positives, alternatively at least about 90% positives, alternatively at least about 91% positives, alternatively at least about 92% positives, alternatively at least about 93% positives, alternatively at least about 94% positives, alternatively at least about 95% positives, alternatively at least about 96% positives, alternatively at least about 97% positives, alternatively at least about 98% positives and alternatively at least about 99% positives when compared with the amino acid sequence of a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein.

15 In a specific aspect, the invention provides an isolated PRO polypeptide without the N-terminal signal sequence and/or the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as hereinbefore described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

25 Another aspect the invention provides an isolated PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

30 In yet another embodiment, the invention concerns agonists and antagonists of a native PRO polypeptide as defined herein. In a particular embodiment, the agonist or antagonist is an anti-PRO antibody or a small molecule.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprise contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native PRO polypeptide.

35 In a still further embodiment, the invention concerns a composition of matter comprising a PRO polypeptide, or an agonist or antagonist of a PRO polypeptide as herein described, or an anti-PRO antibody, in combination with a carrier. Optionally, the carrier is a pharmaceutically acceptable carrier.

40 Another embodiment of the present invention is directed to the use of a PRO polypeptide, or an agonist or antagonist thereof as hereinbefore described, or an anti-PRO antibody, for the preparation of a medicament

useful in the treatment of a condition which is responsive to the PRO polypeptide, an agonist or antagonist thereof or an anti-PRO antibody.

Brief Description of the Drawings

- Figure 1 shows DNA28500 (SEQ ID NO:1).
- 5 Figure 2 shows the native sequence PRO184 polypeptide (SEQ ID NO:2).
- Figure 3 shows DNA30942-1134 (SEQ ID NO:3).
- Figure 4 shows the native sequence PRO212 polypeptide (SEQ ID NO:4).
- Figure 5 shows DNA35638-1141 (SEQ ID NO:8).
- Figure 6 shows the native sequence PRO245 polypeptide (SEQ ID NO:9).
- 10 Figure 7 shows DNA37150-1178 (SEQ ID NO:13).
- Figure 8 shows the native sequence PRO266 polypeptide (SEQ ID NO:14).
- Figure 9 shows DNA39984-1221 (SEQ ID NO:18).
- Figure 10 shows the native sequence PRO306 polypeptide (SEQ ID NO:19).
- Figure 11 shows DNA41374-1312 (SEQ ID NO:26).
- 15 Figure 12 shows the native sequence PRO333 polypeptide (SEQ ID NO:27).
- Figure 13 shows DNA44184-1319 (SEQ ID NO:28).
- Figure 14 shows the native sequence PRO526 polypeptide (SEQ ID NO:29).
- Figure 15 shows DNA44194-1317 (SEQ ID NO:33).
- Figure 16 shows the native sequence PRO381 polypeptide (SEQ ID NO:34).
- 20 Figure 17 shows DNA47365-1206 (SEQ ID NO:38).
- Figure 18 shows the native sequence PRO364 polypeptide (SEQ ID NO:39).
- Figure 19 shows DNA47470-1130 (SEQ ID NO:48).
- Figure 20 shows the native sequence PRO356 polypeptide (SEQ ID NO:49).
- Figure 21 shows DNA49646-1327 (SEQ ID NO:53).
- 25 Figure 22 shows the native sequence PRO719 polypeptide (SEQ ID NO:54).
- Figure 23 shows DNA50798 (SEQ ID NO:58).
- Figure 24 shows the native sequence PRO861 polypeptide (SEQ ID NO:59).
- Figure 25 shows DNA54231-1366 (SEQ ID NO:60).
- Figure 26 shows the native sequence PRO769 polypeptide (SEQ ID NO:61).
- 30 Figure 27 shows DNA56405-1357 (SEQ ID NO:66).
- Figure 28 shows the native sequence PRO788 polypeptide (SEQ ID NO:67).
- Figure 29 shows DNA57694-1341 (SEQ ID NO:68).
- Figure 30 shows the native sequence PRO826 polypeptide (SEQ ID NO:69).
- Figure 31 shows DNA57700-1408 (SEQ ID NO:70).
- 35 Figure 32 shows the native sequence PRO982 polypeptide (SEQ ID NO:71).
- Figure 33 shows DNA58801-1052 (SEQ ID NO:72).
- Figure 34 shows the native sequence PRO779 polypeptide (SEQ ID NO:73).
- Figure 35 shows DNA59214-1449 (SEQ ID NO:76).
- Figure 36 shows the native sequence PRO1068 polypeptide (SEQ ID NO:77).
- 40 Figure 37 shows DNA59294-1381 (SEQ ID NO:78).

Figure 38 shows the native sequence PRO1031 polypeptide (SEQ ID NO:79).  
 Figure 39 shows DNA60292-1506 (SEQ ID NO:80).  
 Figure 40 shows the native sequence PRO1157 polypeptide (SEQ ID NO:81).  
 Figure 41 shows DNA60627-1508 (SEQ ID NO:82).  
 5 Figure 42 shows the native sequence PRO1159 polypeptide (SEQ ID NO:83).  
 Figure 43 shows DNA61185-1646 (SEQ ID NO:84).  
 Figure 44 shows the native sequence PRO1475 polypeptide (SEQ ID NO:85).  
 Figure 45 shows DNA66309-1538-1 (SEQ ID NO:91).  
 Figure 46 shows the native sequence PRO1271 polypeptide (SEQ ID NO:92).  
 10 Figure 47 shows DNA66675-1587 (SEQ ID NO:93).  
 Figure 48 shows the native sequence PRO1343 polypeptide (SEQ ID NO:94).  
 Figure 49 shows DNA67004-1614 (SEQ ID NO:98).  
 Figure 50 shows the native sequence PRO1375 polypeptide (SEQ ID NO:99).  
 Figure 51 shows DNA68864-1629 (SEQ ID NO:100).  
 15 Figure 52 shows the native sequence PRO1418 polypeptide (SEQ ID NO:101).  
 Figure 53 shows DNA73739-1645 (SEQ ID NO:102).  
 Figure 54 shows the native sequence PRO1474 polypeptide (SEQ ID NO:103).  
 Figure 55 shows DNA76400-2528 (SEQ ID NO:104).  
 Figure 56 shows the native sequence PRO1917 polypeptide (SEQ ID NO:105).  
 20 Figure 57 shows DNA82361 (SEQ ID NO:106).  
 Figure 58 shows the native sequence PRO5723 polypeptide (SEQ ID NO:107).  
 Figure 59 shows DNA84920-2614 (SEQ ID NO:108).  
 Figure 60 shows the native sequence PRO4405 polypeptide (SEQ ID NO:109).  
 Figure 61 shows DNA92218-2554 (SEQ ID NO:113).  
 25 Figure 62 shows the native sequence PRO4302 polypeptide (SEQ ID NO:114).  
 Figure 63 shows DNA92282 (SEQ ID NO:115).  
 Figure 64 shows the native sequence PRO9940 polypeptide (SEQ ID NO:116).  
 Figure 65 shows DNA105782-2693 (SEQ ID NO:117).  
 Figure 66 shows the native sequence PRO6006 polypeptide (SEQ ID NO:118).

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#### Detailed Description of the Preferred Embodiments

##### I. Definitions

The term "immune related disease" means a disease in which a component of the immune system of a mammal causes, mediates or otherwise contributes to a morbidity in the mammal. Also included are diseases in  
 35 which stimulation or intervention of the immune response has an ameliorative effect on progression of the disease. Included within this term are immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, etc.

The term "T cell mediated" disease means a disease in which T cells directly or indirectly mediate or otherwise contribute to a morbidity in a mammal. The T cell mediated disease may be associated with cell

mediated effects, lymphokine mediated effects, *etc.*, and even effects associated with B cells if the B cells are stimulated, for example, by the lymphokines secreted by T cells.

Examples of immune-related and inflammatory diseases, some of which are immune or T cell mediated, which can be treated according to the invention include systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis, Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft-versus-host-disease. Infectious diseases including viral diseases such as AIDS (HIV infection), hepatitis A, B, C, D, and E, herpes, *etc.*, bacterial infections, fungal infections, protozoal infections and parasitic infections.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same general structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term "antibody" is used in the broadest sense and specifically covers, without limitation, intact monoclonal antibodies (including agonist, antagonist and neutralizing antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies) formed from at least two intact antibodies, single chain antibodies binding the epitopes specific to the PRO polypeptide and antibody fragments so long as they exhibit the desired biological activity. An anti-PRO antibody is an antibody which immunologically binds to a PRO polypeptide. The antibody may bind to any domain of the PRO polypeptide which may be contacted by the antibody. For example, the antibody may bind to any extracellular domain of the polypeptide and when the entire polypeptide is secreted, to any domain on the polypeptide which is available to the antibody for binding.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain ( $V_H$ ) followed by a number of constant domains. Each light chain has a variable domain at one end ( $V_L$ ) and a constant domain at

its other end: the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three or four segments called "complementarity-determining regions" (CDRs) or "hypervariable regions" in both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four or five FR regions, largely adopting a  $\beta$ -sheet configuration, connected by the CDRs, which form loops connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, NIH Publ. No.91-3242, Vol. I, pages 647-669 (1991)). There are at least two techniques for determining the extent of the CDRs: (1) An approach based on the extent of cross-species sequence variability (*i.e.*, Kabat *et al.*, *Sequences of Proteins of Immunological Interest* (National Institute of Health, Bethesda, MD); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Chothia, C. *et al.*, (1989), *Nature* 342: 877). Moreover, CDR's can also be defined using a hybrid approach incorporating the residues identified by both of the previous techniques. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature*, 256: 495 [1975], or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 [1991] and Marks *et*

*al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example. See also U.S. Patent Nos. 5,750,373, 5,571,698, 5,403,484 and 5,223,409 which describe the preparation of antibodies using phagemid and phage vectors.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 [1984]).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature*, 321:522-525 (1986); Reichmann *et al.*, *Nature*, 332:323-329 [1988]; and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992). The humanized antibody includes a "primatized" antibody where the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest. Antibodies containing residues from Old World monkeys are also possible within the invention. See, for example, U.S. Patent Nos. 5,658,570; 5,693,780; 5,681,722; 5,750,105; and 5,756,096.

Antibodies and fragments thereof in this invention also include "affinity matured" antibodies in which an antibody is altered to change the amino acid sequence of one or more of the CDR regions and/or the framework regions to alter the affinity of the antibody or fragment thereof for the antigen to which it binds. Affinity maturation may result in an increase or in a decrease in the affinity of the matured antibody for the antigen relative to the starting antibody. Typically, the starting antibody will be a humanized, human, chimeric or murine antibody and the affinity matured antibody will have a higher affinity than the starting antibody. During the maturation process, one or more of the amino acid residues in the CDRs or in the framework regions are changed to a different residue using any standard method. Suitable methods include point mutations using well known cassette mutagenesis methods (Wells *et al.*, 1985, *Gene* 34:315) or oligonucleotide mediated mutagenesis methods (Zoller *et al.*, 1987, *Nucleic Acids Res.* 10:6487-6504). Affinity maturation may also be performed using known selection methods in which many mutations are produced and mutants having the

desired affinity are selected from a pool or library of mutants based on improved affinity for the antigen or ligand. Known phage display techniques can be conveniently used in this approach. See, for example, U.S. 5,750,373; U.S. 5,223,409, etc.

Human antibodies are also within the scope of the antibodies of the invention. Human antibodies can be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991); U. S. 5,750, 373]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, *Bio/Technology* 10, 779-783 (1992); Lonberg *et al.*, *Nature* 368 856-859 (1994); Morrison, *Nature* 368, 812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14, 326 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13 65-93 (1995).

The term "effective amount" is at least the minimum concentration or amount of a PRO polypeptide and/or agonist/antagonist which causes, induces or results in either a detectable improvement in a component of the immune response in mammals as measured in an *in vitro* assay. For example, an increase or decrease in the proliferation of T-cells and/or vascular permeability as measured in Examples provided herein. Furthermore, a "therapeutically effective amount" is the minimum concentration or amount of a PRO polypeptide and/or agonist/antagonist which would be effective in at least attenuating a pathology (increasing or decreasing as the case may be) a component of the immune response in mammals, the results of which effects a treatment as defined in the previous paragraph.

The "pathology" of an immune related disease includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth, antibody production, auto-antibody production, complement production and activation, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of any inflammatory or immunological response, infiltration of inflammatory cells (neutrophilic, eosinophilic, monocytic, lymphocytic) into tissue spaces, etc.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g.,  $I^{131}$ ,  $I^{125}$ ,  $Y^{90}$  and  $Re^{186}$ ), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include adriamycin, doxorubicin, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxan, taxoids, e.g., paclitaxel (Taxol, Bristol-Myers Squibb Oncology, Princeton, NJ), and doxetaxel (Taxotere, Rhône-Poulenc Rorer, Antony, France), toxtotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, mitoxantrone,



vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), melphalan and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors such as tamoxifen and onapristone.

- 5 A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially cancer cell overexpressing any of the genes identified herein, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest.
- 10 Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogens, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13.

- The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prolaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- $\alpha$  and - $\beta$ ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- $\beta$ ; platelet-growth factor; transforming growth factors (TGFs) such as
- 25 TGF- $\alpha$  and TGF- $\beta$ ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- $\alpha$ , - $\beta$ , and - $\gamma$ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 $\alpha$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF- $\alpha$  or TNF- $\beta$ ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine
- 30 includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

- "Active" or "activity" in the context of variants of the PRO polypeptide refers to form(s) of proteins of the invention which retain the biologic and/or the ability to induce the production of an antibody against an antigenic epitope possessed by the PRO polypeptide. More specifically, "biological activity" refers to a
- 35 biological function (either inhibitory or stimulatory) caused by a native sequence or naturally-occurring PRO polypeptide. Even more specifically, "biological activity" in the context of an antibody or another molecule that can be identified by the screening assays disclosed herein (*e.g.*, an organic or inorganic small molecule, peptide, *etc.*) can be the ability of such molecules to induce or inhibit infiltration of inflammatory cells into a tissue, to stimulate or inhibit T-cell proliferation or activation, to stimulate or inhibit cytokine release by cells or to
- 40 increase or decrease vascular permeability. Another specific biological activity is the increased vascular

permeability or the inhibition thereof.

## II. Additional Definitions

The terms "PRO polypeptide" and "PRO" as used herein and when immediately followed by a numerical designation refer to various polypeptides, wherein the complete designation (*i.e.*, PRO/number) refers to specific polypeptide sequences as described herein. The terms "PRO/number polypeptide" and "PRO/number" wherein the term "number" is provided as an actual numerical designation as used herein encompass native sequence polypeptides and polypeptide variants (which are further defined herein). The PRO polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods. The term "PRO polypeptide" refers to each individual PRO/number polypeptide disclosed herein. All disclosures in this specification which refer to the "PRO polypeptide" refer to each of the polypeptides individually as well as jointly. For example, descriptions of the preparation of, purification of, derivation of, formation of antibodies to or against, administration of, compositions containing, treatment of a disease with, etc., pertain to each polypeptide of the invention individually. The term "PRO polypeptide" also includes variants of the PRO/number polypeptides disclosed herein.

A "native sequence PRO polypeptide" comprises a polypeptide having the same amino acid sequence as the corresponding PRO polypeptide derived from nature. Such native sequence PRO polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence PRO polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of the specific PRO polypeptide (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. In various embodiments of the invention, the native sequence PRO polypeptides disclosed herein are mature or full-length native sequence polypeptides comprising the full-length amino acids sequences shown in the accompanying figures. Start and stop codons are shown in bold font and underlined in the figures. However, while the PRO polypeptide disclosed in the accompanying figures are shown to begin with methionine residues designated herein as amino acid position 1 in the figures, it is conceivable and possible that other methionine residues located either upstream or downstream from the amino acid position 1 in the figures may be employed as the starting amino acid residue for the PRO polypeptides.

The PRO polypeptide "extracellular domain" or "ECD" refers to a form of the PRO polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a PRO polypeptide ECD will have less than 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5% of such domains. It will be understood that any transmembrane domains identified for the PRO polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified herein. Optionally, therefore, an extracellular domain of a PRO polypeptide may contain from about 5 or fewer amino acids on either side of the transmembrane domain/extracellular domain boundary as identified in the Examples or specification and such polypeptides, with or without the associated signal peptide, and nucleic acid encoding them, are contemplated by the present invention.

The approximate location of the "signal peptides" of the various PRO polypeptides disclosed herein are shown in the present specification and/or the accompanying figures. It is noted, however, that the C-terminal boundary of a signal peptide may vary, but most likely by no more than about 5 amino acids on either side of the signal peptide C-terminal boundary as initially identified herein, wherein the C-terminal boundary of the signal peptide may be identified pursuant to criteria routinely employed in the art for identifying that type of amino acid sequence element (e.g., Nielsen *et al.*, *Prot. Eng.* 10:1-6 (1997) and von Heinje *et al.*, *Nucl. Acids. Res.* 14:4683-4690 (1986)). Moreover, it is also recognized that, in some cases, cleavage of a signal sequence from a secreted polypeptide is not entirely uniform, resulting in more than one secreted species. These mature polypeptides, where the signal peptide is cleaved within no more than about 5 amino acids on either side of the C-terminal boundary of the signal peptide as identified herein, and the polynucleotides encoding them, are contemplated by the present invention.

"PRO polypeptide variant" means an active PRO polypeptide as defined above or below having at least about 80% amino acid sequence identity with a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Such PRO polypeptide variants include, for instance, PRO polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the full-length native amino acid sequence. Ordinarily, a PRO polypeptide variant will have at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, PRO variant polypeptides are at least about 10 amino acids in length, alternatively at least about 20 amino acids in length, alternatively at least about 30 amino acids in length, alternatively at least about 40 amino acids in length, alternatively at least about 50 amino acids in length, alternatively at least about 60 amino acids in length, alternatively at least about 70 amino acids in length, alternatively at least about 80 amino acids in length, alternatively at least about 90 amino acids in length, alternatively at least about 100 amino acids in length, alternatively at least about 150 amino acids in length, alternatively at least about 200 amino acids in length, alternatively at least about 300 amino acids in length, or more.

"Percent (%) amino acid sequence identity" with respect to the PRO polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific PRO polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. As examples of % amino acid sequence identity calculations using this method, Tables 2 and 3 demonstrate how to calculate the % amino acid sequence identity of the amino-acid sequence designated "Comparison Protein" to the amino acid sequence designated "PRO", wherein "PRO" represents the amino acid sequence of a hypothetical PRO polypeptide of interest, "Comparison Protein" represents the amino acid sequence of a polypeptide against which the "PRO" polypeptide of interest is being compared, and "X," "Y" and "Z" each represent different hypothetical amino acid residues.

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % amino acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, *i.e.*, the adjustable

parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the PRO polypeptide of interest having a sequence derived from the native PRO polypeptide and the comparison amino acid sequence of interest (i.e., the sequence against which the PRO polypeptide of interest is being compared which may be a PRO variant polypeptide) as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest. For example, in the statement "a polypeptide comprising an the amino acid sequence A which has or having at least 80% amino acid sequence identity to the amino acid sequence B", the amino acid sequence A is the comparison amino acid sequence of interest and the amino acid sequence B is the amino acid sequence of the PRO polypeptide of interest.

Percent amino acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction  $X/Y$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

"PRO variant polynucleotide" or "PRO variant nucleic acid sequence" means a nucleic acid molecule which encodes an active PRO polypeptide as defined below and which has at least about 80% nucleic acid sequence identity with a nucleotide acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, a PRO variant polynucleotide will have at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence

identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity with a nucleic acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal sequence, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Variants do not encompass the native nucleotide sequence.

Ordinarily, PRO variant polynucleotides are at least about 30 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 210 nucleotides in length, alternatively at least about 240 nucleotides in length, alternatively at least about 270 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 900 nucleotides in length, or more.

"Percent (%) nucleic acid sequence identity" with respect to PRO-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the PRO nucleic acid sequence of interest, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. For purposes herein, however, % nucleic acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for nucleic acid sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

100 times the fraction  $W/Z$

where W is the number of nucleotides scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C. As examples of % nucleic acid sequence identity calculations, Tables 4 and 5, demonstrate how to calculate the % nucleic acid sequence identity of the nucleic acid sequence designated "Comparison DNA" to the nucleic acid sequence designated "PRO-DNA", wherein "PRO-DNA" represents a hypothetical PRO-encoding nucleic acid sequence of interest, "Comparison DNA" represents the nucleotide sequence of a nucleic acid molecule against which the "PRO-DNA" nucleic acid molecule of interest is being compared, and "N", "L" and "V" each represent different hypothetical nucleotides.

Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % nucleic acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest having a sequence derived from the native sequence PRO polypeptide-encoding nucleic acid and the comparison nucleic acid molecule of interest (i.e., the sequence against which the PRO polypeptide-encoding nucleic acid molecule of interest is being compared which may be a variant PRO polynucleotide) as determined by WU-BLAST-2 by (b) the total number of nucleotides of the PRO polypeptide-encoding nucleic acid molecule of interest. For example, in the statement "an isolated nucleic acid molecule comprising a nucleic acid sequence A which has or having at least 80% nucleic acid sequence identity to the nucleic acid sequence B", the nucleic acid sequence A is the comparison nucleic acid molecule of interest and the nucleic acid sequence B is the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest.

Percent nucleic acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

100 times the fraction  $W/Z$

where  $W$  is the number of nucleotides scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of  $C$  and  $D$ , and where  $Z$  is the total number of nucleotides in  $D$ . It will be appreciated that where the length of nucleic acid sequence  $C$  is not equal to the length of nucleic acid sequence  $D$ , the % nucleic acid sequence identity of  $C$  to  $D$  will not equal the % nucleic acid sequence identity of  $D$  to  $C$ .

In other embodiments, PRO variant polynucleotides are nucleic acid molecules that encode an active PRO polypeptide and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding a full-length PRO polypeptide as disclosed herein. PRO variant polypeptides may be those that are encoded by a PRO variant polynucleotide.

The term "positives", in the context of sequence comparison performed as described above, includes residues in the sequences compared that are not identical but have similar properties (e.g. as a result of conservative substitutions, see Table 6 below). For purposes herein, the % value of positives is determined by dividing (a) the number of amino acid residues scoring a positive value between the PRO polypeptide amino acid sequence of interest having a sequence derived from the native PRO polypeptide sequence and the comparison amino acid sequence of interest (i.e., the amino acid sequence against which the PRO polypeptide sequence is being compared) as determined in the BLOSUM62 matrix of WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest.

Unless specifically stated otherwise, the % value of positives is calculated as described in the immediately preceding paragraph. However, in the context of the amino acid sequence identity comparisons performed as described for ALIGN-2 and NCBI-BLAST-2 above, includes amino acid residues in the sequences compared that are not only identical, but also those that have similar properties. Amino acid residues that score a positive value to an amino acid residue of interest are those that are either identical to the amino acid residue of interest or are a preferred substitution (as defined in Table 6 below) of the amino acid residue of interest.

For amino acid sequence comparisons using ALIGN-2 or NCBI-BLAST2, the % value of positives of a given amino acid sequence  $A$  to, with, or against a given amino acid sequence  $B$  (which can alternatively be phrased as a given amino acid sequence  $A$  that has or comprises a certain % positives to, with, or against a given amino acid sequence  $B$ ) is calculated as follows:

100 times the fraction  $X/Y$

where  $X$  is the number of amino acid residues scoring a positive value as defined above by the sequence alignment program ALIGN-2 or NCBI-BLAST2 in that program's alignment of  $A$  and  $B$ , and where  $Y$  is the total number of amino acid residues in  $B$ . It will be appreciated that where the length of amino acid sequence  $A$  is not equal to the length of amino acid sequence  $B$ , the % positives of  $A$  to  $B$  will not equal the % positives of  $B$  to  $A$ .

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic



uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" PRO polypeptide-encoding nucleic acid or other polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. An isolated polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated polypeptide-encoding nucleic acid molecules therefore are distinguished from the specific polypeptide-encoding nucleic acid molecule as it exists in natural cells. However, an isolated polypeptide-encoding nucleic acid molecule includes polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express the polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "antibody" is used in the broadest sense and specifically covers, for example, single anti-PRO monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-PRO antibody compositions with polypeptidic specificity, single chain anti-PRO antibodies, and fragments of anti-PRO antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of

desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

"Active" or "activity" for the purposes herein refers to form(s) of a PRO polypeptide which retain a biological and/or an immunological activity of native or naturally-occurring PRO, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring PRO other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO polypeptides, peptides, antisense oligonucleotides, small organic molecules, etc. Methods for identifying agonists or antagonists of a PRO polypeptide may comprise contacting a PRO polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the PRO polypeptide.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN<sup>TM</sup>, polyethylene glycol (PEG), and PLURONICS<sup>TM</sup>.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv

fragments; diabodies; linear antibodies (Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an  $F(ab')_2$  fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the  $V_H$ - $V_L$  dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group.  $F(ab')_2$  antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

"Single-chain Fv" or "sFv" antibody fragments comprise the  $V_H$  and  $V_L$  domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the  $V_H$  and  $V_L$  domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain ( $V_H$ ) connected to a light-chain variable domain ( $V_L$ ) in the same polypeptide chain ( $V_H$ - $V_L$ ). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to

greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a PRO polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

Table 1

```

/*
 *
 * C-C increased from 12 to 15
5  * Z is average of EQ
 * B is average of ND
 * match with stop is _M: stop-stop = 0; J (joker) match = 0
 */
#define _M -8 /* value of a match with a stop */
10 int _day[26][26] = {
/* A B C D E F G H I J K L M N O P Q R S T U V W X Y Z */
/* A */ { 2, 0, -2, 0, 0, -4, 1, -1, -1, 0, -1, -2, -1, 0, _M, 1, 0, -2, 1, 1, 0, 0, -6, 0, -3, 0},
/* B */ { 0, 3, -4, 3, 2, -5, 0, 1, -2, 0, 0, -3, -2, 2, _M, -1, 1, 0, 0, 0, 0, -2, -5, 0, -3, 1},
15 /* C */ {-2, -4, 15, -5, -5, -4, -3, -3, -2, 0, -5, -6, -5, -4, _M, -3, -5, -4, 0, -2, 0, -2, -8, 0, 0, -5},
/* D */ { 0, 3, -5, 4, 3, -6, 1, 1, -2, 0, 0, -4, -3, 2, _M, -1, 2, -1, 0, 0, 0, -2, -7, 0, -4, 2},
/* E */ { 0, 2, -5, 3, 4, -5, 0, 1, -2, 0, 0, -3, -2, 1, _M, -1, 2, -1, 0, 0, 0, -2, -7, 0, -4, 3},
/* F */ {-4, -5, -4, -6, -5, 9, -5, -2, 1, 0, -5, 2, 0, -4, _M, -5, -5, -4, -3, -3, 0, -1, 0, 0, 7, -5},
/* G */ { 1, 0, -3, 1, 0, -5, 5, -2, -3, 0, -2, -4, -3, 0, _M, -1, -1, -3, 1, 0, 0, -1, -7, 0, -5, 0},
20 /* H */ {-1, 1, -3, 1, 1, -2, -2, 6, -2, 0, 0, -2, -2, 2, _M, 0, 3, 2, -1, -1, 0, -2, -3, 0, 0, 2},
/* I */ {-1, -2, -2, -2, 1, -3, -2, 5, 0, -2, 2, 2, -2, _M, -2, -2, -2, -1, 0, 0, 4, -5, 0, -1, -2},
/* J */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */ {-1, 0, -5, 0, 0, -5, -2, 0, -2, 0, 5, -3, 0, 1, _M, -1, 1, 3, 0, 0, 0, -2, -3, 0, -4, 0},
/* L */ {-2, -3, -6, -4, -3, 2, -4, -2, 2, 0, -3, 6, 4, -3, _M, -3, -2, -3, -3, -1, 0, 2, -2, 0, -1, -2},
25 /* M */ {-1, -2, -5, -3, -2, 0, -3, -2, 2, 0, 0, 4, 6, -2, _M, -2, -1, 0, -2, -1, 0, 2, -4, 0, -2, -1},
/* N */ { 0, 2, -4, 2, 1, -4, 0, 2, -2, 0, 1, -3, -2, 2, _M, -1, 1, 0, 1, 0, 0, -2, -4, 0, -2, 1},
/* O */ {_M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, 0, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M},
/* P */ { 1, -1, -3, -1, -1, -5, -1, 0, -2, 0, -1, -3, -2, -1, _M, 6, 0, 0, 1, 0, 0, -1, -6, 0, -5, 0},
/* Q */ { 0, 1, -5, 2, 2, -5, -1, 3, -2, 0, 1, -2, -1, 1, _M, 0, 4, 1, -1, -1, 0, -2, -5, 0, -4, 3},
30 /* R */ {-2, 0, -4, -1, -1, -4, -3, 2, -2, 0, 3, -3, 0, 0, _M, 0, 1, 6, 0, -1, 0, -2, 2, 0, -4, 0},
/* S */ { 1, 0, 0, 0, 0, -3, 1, -1, -1, 0, 0, -3, -2, 1, _M, 1, -1, 0, 2, 1, 0, -1, -2, 0, -3, 0},
/* T */ { 1, 0, -2, 0, 0, -3, 0, -1, 0, 0, 0, -1, -1, 0, _M, 0, -1, -1, 1, 3, 0, 0, -5, 0, -3, 0},
/* U */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */ { 0, -2, -2, -2, -1, -1, -2, 4, 0, -2, 2, 2, -2, _M, -1, -2, -2, -1, 0, 0, 4, -6, 0, -2, -2},
35 /* W */ {-6, -5, -8, -7, -7, 0, -7, -3, -5, 0, -3, -2, -4, -4, _M, -6, -5, 2, -2, -5, 0, -6, 17, 0, 0, -6},
/* X */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */ {-3, -3, 0, -4, -4, 7, -5, 0, -1, 0, -4, -1, -2, -2, _M, -5, -4, -4, -3, -3, 0, -2, 0, 0, 10, -4},
/* Z */ { 0, 1, -5, 2, 3, -5, 0, 2, -2, 0, 0, -2, -1, 1, _M, 0, 3, 0, 0, 0, 0, -2, -6, 0, -4, 4}
};
40
45
50

```

Table 1 (cont')

```

/*
*/
#include <stdio.h>
5  #include <ctype.h>

#define MAXJMP      16      /* max jumps in a diag */
#define MAXGAP      24      /* don't continue to penalize gaps larger than this */
#define JMPS        1024    /* max jmps in an path */
10  #define MX        4       /* save if there's at least MX-1 bases since last jmp */

#define DMAT         3      /* value of matching bases */
#define DMIS         0      /* penalty for mismatched bases */
#define DINSO         8      /* penalty for a gap */
15  #define DINSI         1    /* penalty per base */
#define PINSO         8      /* penalty for a gap */
#define PINSI         4      /* penalty per residue */

struct jmp {
20      short          n[MAXJMP]; /* size of jmp (neg for dely) */
      unsigned short  x[MAXJMP]; /* base no. of jmp in seq x */
}; /* limits seq to 2^16 -1 */

struct diag {
25      int            score; /* score at last jmp */
      long            offset; /* offset of prev block */
      short           ijmp; /* current jmp index */
      struct jmp      jp; /* list of jmps */
};
30

struct path {
      int             spc; /* number of leading spaces */
      short           n[JMPS]; /* size of jmp (gap) */
      int             x[JMPS]; /* loc of jmp (last elem before gap) */
35  };

char      *ofile; /* output file name */
char      *namex[2]; /* seq names: getseqs() */
char      *prog; /* prog name for err msgs */
40  char      *seqx[2]; /* seqs: getseqs() */
int       dmax; /* best diag: nw() */
int       dmax0; /* final diag */
int       dna; /* set if dna: main() */
int       endgaps; /* set if penalizing end gaps */
45  int       gapx, gapy; /* total gaps in seqs */
int       len0, len1; /* seq lens */
int       ngapx, ngapy; /* total size of gaps */
int       smax; /* max score: nw() */
int       *xbm; /* bitmap for matching */
50  long      offset; /* current offset in jmp file */
struct     diag *dx; /* holds diagonals */
struct     path pp[2]; /* holds path for seqs */

char      *calloc(), *malloc(), *index(), *strcpy();
55  char      *getseq(), *g_calloc();

```

60

Table 1 (cont')

```

/* Needleman-Wunsch alignment program
*
* usage: prog file1 file2
* where file1 and file2 are two dna or two protein sequences.
* The sequences can be in upper- or lower-case and may contain ambiguity
* Any lines beginning with ':', '>' or '<' are ignored
* Max file length is 65535 (limited by unsigned short x in the jmp struct)
* A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
* Output is in the file "align.out"
*
* The program may create a tmp file in /tmp to hold info about traceback.
* Original version developed under BSD 4.3 on a vax 8650
*/
#include "nw.h"
#include "day.h"

static _dbval[26] = {
    1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static _pbval[26] = {
    1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
    128, 256, 0xFFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
    1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
    1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

main(ac, av)                                main
{
    int      ac;
    char     *av[];

    prog = av[0];
    if (ac != 3) {
        fprintf(stderr, "usage: %s file1 file2\n", prog);
        fprintf(stderr, "where file1 and file2 are two dna or two protein sequences.\n");
        fprintf(stderr, "The sequences can be in upper- or lower-case\n");
        fprintf(stderr, "Any lines beginning with ':', '>' or '<' are ignored\n");
        fprintf(stderr, "Output is in the file \"align.out\"\n");
        exit(1);
    }
    namex[0] = av[1];
    namex[1] = av[2];
    seqx[0] = getseq(namex[0], &len0);
    seqx[1] = getseq(namex[1], &len1);
    xbm = (dna)? _dbval : _pbval;

    endgaps = 0;                                /* 1 to penalize endgaps */
    ofile = "align.out";                        /* output file */

    nw();                                        /* fill in the matrix, get the possible jumps */
    readjumps();                                /* get the actual jumps */
    print();                                    /* print stats; alignment */

    cleanup(0);                                /* unlink any tmp files */
}

```



Table 1 (cont')

```

/* do the alignment, return best score: main()
 * dna: values in Fitch and Smith. PNAS. 80. 1382-1386. 1983
 * pro: PAM 250 values
5  * When scores are equal, we prefer mismatches to any gap. prefer
 * a new gap to extending an ongoing gap. and prefer a gap in seqx
 * to a gap in seq y.
 */
nw()
10 {
    char      *px, *py;      /* seqs and ptrs */
    int       *ndely, *dely; /* keep track of dely */
    int       ndelx, delx;   /* keep track of delx */
    int       *tmp;         /* for swapping row0, row1 */
15    int       mis;          /* score for each type */
    int       ins0, ins1;    /* insertion penalties */
    register  id;           /* diagonal index */
    register  ij;           /* jmp index */
    register  *col0, *col1; /* score for curr. last row */
20    register  xx, yy;       /* index into seqs */

    dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));

    ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
25    dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
    col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
    col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
    ins0 = (dna)? DINS0 : PINS0;
    ins1 = (dna)? DINS1 : PINS1;

30    smax = -10000;
    if (endgaps) {
        for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
            col0[yy] = dely[yy] = col0[yy-1] - ins1;
            ndely[yy] = yy;
35        }
        col0[0] = 0; /* Waterman Bull Math Biol 84 */
    }
    else
40        for (yy = 1; yy <= len1; yy++)
            dely[yy] = -ins0;

    /* fill in match matrix
    */
45    for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
        /* initialize first entry in col
        */
        if (endgaps) {
            if (xx == 1)
50                col1[0] = delx = -(ins0+ins1);
            else
                col1[0] = delx = col0[0] - ins1;
            ndelx = xx;
        }
        else {
55            col1[0] = 0;
            delx = -ins0;
            ndelx = 0;
        }
    }
60

```

nw

Table 1 (cont')

...nw

```

5   for (py = seqx[l], yy = 1; yy <= len1; py++, yy++) {
      mis = col0[yy-1];
      if (dna)
          mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
      else
          mis += _day[*px-'A'][*py-'A'];

10  /* update penalty for del in x seq;
      * favor new del over ongoing del
      * ignore MAXGAP if weighting endgaps
      */
      if (endgaps || ndely[yy] < MAXGAP) {
15  if (col0[yy] - ins0 >= dely[yy]) {
          dely[yy] = col0[yy] - (ins0+ins1);
          ndely[yy] = 1;
        } else {
          dely[yy] -= ins1;
          ndely[yy]++;
20  }
      } else {
          if (col0[yy] - (ins0+ins1) >= dely[yy]) {
25  dely[yy] = col0[yy] - (ins0+ins1);
          ndely[yy] = 1;
          } else
              ndely[yy]++;
      }

30  /* update penalty for del in y seq;
      * favor new del over ongoing del
      */
      if (endgaps || ndelx < MAXGAP) {
35  if (col1[yy-1] - ins0 >= delx) {
          delx = col1[yy-1] - (ins0+ins1);
          ndelx = 1;
        } else {
          delx -= ins1;
          ndelx++;
40  }
      } else {
          if (col1[yy-1] - (ins0+ins1) >= delx) {
45  delx = col1[yy-1] - (ins0+ins1);
          ndelx = 1;
          } else
              ndelx++;
      }

50  /* pick the maximum score: we're favoring
      * mis over any del and delx over dely
      */

```

55

60

Table 1 (cont')

```

id = xx - yy + len1 - 1;
if (mis >= delx && mis >= dely[yy])
    coll[yy] = mis;
5   else if (delx >= dely[yy]) {
    coll[yy] = delx;
    ij = dx[id].ijmp;
    if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
10   && xx > dx[id].jp.x[ij] + MX) || mis > dx[id].score + DINSO)) {
        dx[id].ijmp++;
        if (++ij >= MAXJMP) {
            writejmps(id);
            ij = dx[id].ijmp = 0;
            dx[id].offset = offset;
            offset += sizeof(struct jmp) + sizeof(offset);
15   }
        }
        dx[id].jp.n[ij] = ndelx;
        dx[id].jp.x[ij] = xx;
        dx[id].score = delx;
20   }
    else {
        coll[yy] = dely[yy];
        ij = dx[id].ijmp;
25   if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
        && xx > dx[id].jp.x[ij] + MX) || mis > dx[id].score + DINSO)) {
            dx[id].ijmp++;
            if (++ij >= MAXJMP) {
                writejmps(id);
                ij = dx[id].ijmp = 0;
                dx[id].offset = offset;
                offset += sizeof(struct jmp) + sizeof(offset);
30   }
            }
            dx[id].jp.n[ij] = -ndely[yy];
            dx[id].jp.x[ij] = xx;
            dx[id].score = dely[yy];
35   }
        if (xx == len0 && yy < len1) {
            /* last col
            */
            if (endgaps)
                coll[yy] -= ins0 + ins1 * (len1 - yy);
            if (coll[yy] > smax) {
                smax = coll[yy];
                dmax = id;
45   }
        }
    }
}
50   if (endgaps && xx < len0)
        coll[yy-1] -= ins0 + ins1 * (len0 - xx);
    if (coll[yy-1] > smax) {
        smax = coll[yy-1];
        dmax = id;
55   }
    ump = col0; col0 = coll; coll = ump;
}
(void) free((char *)ndely);
(void) free((char *)dely);
60   (void) free((char *)col0);
    (void) free((char *)coll);
}

```

Table 1 (cont')

```

/*
 *
 * print() -- only routine visible outside this module
 *
 * static:
 * getmat() -- trace back best path. count matches: print()
 * pr_align() -- print alignment of described in array p[]: print()
 * dumpblock() -- dump a block of lines with numbers. stars: pr_align()
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() -- put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */

#include "nw.h"

#define SPC      3
#define P_LINE  256 /* maximum output line */
#define P_SPC    3 /* space between name or num and seq */

extern _day[26][26];
int olen; /* set output line length */
FILE *fx; /* output file */

print()
{
    int lx, ly, firstgap, lastgap; /* overlap */

    if ((fx = fopen(ofile, "w")) == 0) {
        fprintf(stderr, "%s: can't write %s\n", prog, ofile);
        cleanup(1);
    }
    fprintf(fx, "< first sequence: %s (length = %d)\n", namex[0], len0);
    fprintf(fx, "< second sequence: %s (length = %d)\n", namex[1], len1);
    olen = 60;
    lx = len0;
    ly = len1;
    firstgap = lastgap = 0;
    if (dmax < len1 - 1) { /* leading gap in x */
        pp[0].spc = firstgap = len1 - dmax - 1;
        ly -= pp[0].spc;
    }
    else if (dmax > len1 - 1) { /* leading gap in y */
        pp[1].spc = firstgap = dmax - (len1 - 1);
        lx -= pp[1].spc;
    }
    if (dmax0 < len0 - 1) { /* trailing gap in x */
        lastgap = len0 - dmax0 - 1;
        lx -= lastgap;
    }
    else if (dmax0 > len0 - 1) { /* trailing gap in y */
        lastgap = dmax0 - (len0 - 1);
        ly -= lastgap;
    }
    getmat(lx, ly, firstgap, lastgap);
    pr_align();
}

```

Table 1 (cont')

```

/*
 * trace back the best path. count matches
 */
5 static
  getmat(lx, ly, firstgap, lastgap)                                getmat
      int    lx, ly;                                           /* "core" (minus endgaps) */
      int    firstgap, lastgap;                                /* leading trailing overlap */
{
10     int      nm, i0, i1, siz0, siz1;
      char     outx[32];
      double   pct;
      register n0, n1;
      register char *p0, *p1;

15     /* get total matches. score
      */
      i0 = i1 = siz0 = siz1 = 0;
      p0 = seqx[0] + pp[1].spc;
20     p1 = seqx[1] + pp[0].spc;
      n0 = pp[1].spc + 1;
      n1 = pp[0].spc + 1;

      nm = 0;
25     while ( *p0 && *p1 ) {
          if (siz0) {
              p1++;
              n1++;
              siz0--;
30           }
          else if (siz1) {
              p0++;
              n0++;
              siz1--;
35           }
          else {
              if (xbm[*p0-'A']&xbm[*p1-'A'])
                  nm++;
              if (n0++ == pp[0].x[i0])
                  siz0 = pp[0].n[i0++];
40             if (n1++ == pp[1].x[i1])
                  siz1 = pp[1].n[i1++];
              p0++;
              p1++;
45           }
      }

      /* pct homology:
      * if penalizing endgaps, base is the shorter seq
      * else, knock off overhangs and take shorter core
      */
      if (endgaps)
          lx = (len0 < len1)? len0 : len1;
      else
55         lx = (lx < ly)? lx : ly;
      pct = 100.*((double)nm)/((double)lx);
      fprintf(fx, "\n");
      fprintf(fx, "< %d match %s in an overlap of %d: %.2f percent similarity\n",
60             nm, (nm == 1)? "" : "es", lx, pct);

```

Table 1 (cont')

```

fprintf(fx, "< gaps in first sequence: %d", gapx);
if (gapx) {
    (void) sprintf(oux, " (%d %s%s)",
        ngapx, (dna)? "base": "residue", (ngapx == 1)? "": "s");
    fprintf(fx, "%s", oux);

    fprintf(fx, ". gaps in second sequence: %d", gapy);
    if (gapy) {
        (void) sprintf(oux, " (%d %s%s)",
            ngapy, (dna)? "base": "residue", (ngapy == 1)? "": "s");
        fprintf(fx, "%s", oux);
    }
    if (dna)
        fprintf(fx,
            "\n< score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
            smax, DMAT, DMIS, DINS0, DINS1);
    else
        fprintf(fx,
            "\n< score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
            smax, PINS0, PINS1);
    if (endgaps)
        fprintf(fx,
            "< endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
            firstgap, (dna)? "base": "residue", (firstgap == 1)? "": "s",
            lastgap, (dna)? "base": "residue", (lastgap == 1)? "": "s");
    else
        fprintf(fx, "< endgaps not penalized\n");
}

static nm; /* matches in core -- for checking */
static lmax; /* lengths of stripped file names */
static ij[2]; /* jmp index for a path */
static nc[2]; /* number at start of current line */
static ni[2]; /* current elem number -- for gapping */
static siz[2];
static char *ps[2]; /* ptr to current element */
static char *po[2]; /* ptr to next output char slot */
static char out[2][P_LINE]; /* output line */
static char star[P_LINE]; /* set by stars() */

/*
 * print alignment of described in struct path pp[]
 */
static
pr_align()
{
    int nn; /* char count */
    int more;
    register i;

    for (i = 0, lmax = 0; i < 2; i++) {
        nn = strippname(name[i]);
        if (nn > lmax)
            lmax = nn;

        nc[i] = 1;
        ni[i] = 1;
        siz[i] = ij[i] = 0;
        ps[i] = seqx[i];
        po[i] = our[i];
    }
}

```

...getmat

pr\_align

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Table 1 (cont')

```

for (nn = nm = 0, more = 1; more;) {
    for (i = more = 0; i < 2; i++) {
        /*
5         * do we have more of this sequence?
        */
        if (!*ps[i])
            continue;

10        more++;

        if (pp[i].spc) { /* leading space */
            *po[i]++ = ' ';
            pp[i].spc--;
15        }
        else if (siz[i]) { /* in a gap */
            *po[i]++ = '-';
            siz[i]--;
20        }
        else { /* we're putting a seq element
            */
            *po[i] = *ps[i];
            if (islower(*ps[i]))
                *ps[i] = toupper(*ps[i]);
25            po[i]++;
            ps[i]++;

            /*
            * are we at next gap for this seq?
            */
30            if (ni[i] == pp[i].x[ij[i]]) {
                /*
                * we need to merge all gaps
                * at this location
                */
35                siz[i] = pp[i].n[ij[i] + +];
                while (ni[i] == pp[i].x[ij[i]])
                    siz[i] += pp[i].n[ij[i] + +];
            }
            ni[i]++;
40        }
    }
    if (++nn == olen || !more && nn) {
        dumpblock();
        for (i = 0; i < 2; i++)
            po[i] = out[i];
        nn = 0;
45    }
}

/*
 * dump a block of lines, including numbers, stars: pr_align()
 */
55 static
dumpblock()
{
    register i;

60    for (i = 0; i < 2; i++)
        *po[i]-- = '\0';
}

```

dumpblock

Table 1 (cont')

...dumpblock

```

5      (void) puts("\n", fx);
      for (i = 0; i < 2; i++) {
          if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
              if (i == 0)
                  nums(i);
              if (i == 0 && *out[1])
                  stars();
10         putline(i);
              if (i == 0 && *out[1])
                  fprintf(fx, star);
              if (i == 1)
                  nums(i);
15         }
      }
  }

20  /*
   * put out a number line: dumpblock()
   */
   static
   nums(ix)
25     int    ix;      /* index in out[] holding seq line */
   {
       char    nline[P_LINE];
       register i, j;
       register char *pn, *px, *py;
30     for (pn = nline, i = 0; i < lmax + P_SPC; i++, pn++)
           *pn = ' ';
       for (i = nc[ix], py = out[ix]; *py: py++, pn++) {
           if (*py == ' ' || *py == '-')
35             *pn = ' ';
           else {
               if (i % 10 == 0 || (i == 1 && nc[ix] != 1)) {
                   j = (i < 0)? -i : i;
                   for (px = pn; j /= 10, px--)
40                     *px = j % 10 + '0';
                   if (i < 0)
                       *px = '-';
               }
               else
45                 *pn = ' ';
               i++;
           }
       }
       *pn = '\0';
       nc[ix] = i;
       for (pn = nline; *pn: pn++)
           (void) puts(*pn, fx);
       (void) puts("\n", fx);
55  }

   /*
   * put out a line (name, [num], seq. [num]): dumpblock()
   */
   static
   putline(ix)
60     int    ix;
   {

```

putline

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Table 1 (cont')

...putline

```

5      int          i;
      register char *px;

      for (px = namex[ix], i = 0; *px && *px != ' '; px++, i++)
          (void)putc(*px, fx);
      for (; i < lmax + P_SPC; i++)
10         (void)putc(' ', fx);

      /* these count from 1:
       * ni[] is current element (from 1)
       * nc[] is number at start of current line
       */
15     for (px = out[ix]; *px; px++)
        (void)putc(*px & 0x7F, fx);
      (void)putc('\n', fx);
20 }

/*
 * put a line of stars (seqs always in out[0], out[1]); dumpblock()
 */
25 static
stars()
{
    int          i;
    register char *p0, *p1, cx, *px;

30     if (!*out[0] || (*out[0] == ' ' && *out[0] == ' ') ||
        !*out[1] || (*out[1] == ' ' && *out[1] == ' '))
        return;
    px = star;
35     for (i = lmax + P_SPC; i; i--)
        *px++ = ' ';

    for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
40         if (isalpha(*p0) && isalpha(*p1)) {
            cx = '*';
            nm++;
        }
45         else if (!dna && day[*p0-'A'][*p1-'A'] > 0)
            cx = '.';
        else
            cx = ' ';

50         }
        else
            cx = ' ';
        *px++ = cx;
    }
    *px++ = '\n';
55     *px = '\0';
}

```

stars

Table 1 (cont')

```

/*
 * strip path or prefix from pn. return len: pr_align()
 */
5 static
stripname(pn)                                stripname
    char    *pn;    /* file name (may be path) */
{
    register char    *px, *py;
10
    py = 0;
    for (px = pn; px++; )
        if (*px == '/')
            py = px + 1;
15
    if (py)
        (void) strcpy(pn, py);
    return(strlen(pn));
20
}
25
30
35
40
45
50
55
60

```

Table 1 (cont')

```

/*
 * cleanup() -- cleanup any tmp file
 * getseq() -- read in seq. set dna. len. maxlen
5  * g_calloc() -- calloc() with error checkin
 * readjumps() -- get the good jumps. from tmp file if necessary
 * writejumps() -- write a filled array of jumps to a tmp file: nw()
 */
#include "nw.h"
10 #include <sys/file.h>

char *jname = "/tmp/homg.XXXXXX"; /* tmp file for jumps */
FILE *fj;

15 int cleanup(); /* cleanup tmp file */
long lseek();

/*
 * remove any tmp file if we blow
20 */
cleanup(i)
    int i;
{
    if (fj)
25     (void) unlink(jname);
    exit(i);
}

/*
30 * read, return ptr to seq, set dna. len. maxlen
 * skip lines starting with '.', '<', or '>'
 * seq in upper or lower case
 */
char *
35 getseq(file, len)
    char *file; /* file name */
    int *len; /* seq len */
{
    char line[1024], *pseq;
    register char *px, *py;
    int natgc, den;
    FILE *fp;

    if ((fp = fopen(file, "r")) == 0) {
45         fprintf(stderr, "%s: can't read %s\n", prog, file);
        exit(1);
    }
    den = natgc = 0;
    while (fgets(line, 1024, fp)) {
50         if (*line == '.' || *line == '<' || *line == '>')
            continue;
        for (px = line; *px != '\n'; px++)
            if (isupper(*px) || islower(*px))
                den++;
55     }
    if ((pseq = malloc((unsigned)(den+6))) == 0) {
        fprintf(stderr, "%s: malloc() failed to get %d bytes for %s\n", prog, den+6, file);
        exit(1);
    }
    pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';
60

```

Table 1 (cont')

...getseq

```

py = pseq + 4;
*len = llen;
rewind(fp);

while (fgets(line, 1024, fp)) {
    if (*line == ':' || *line == '<' || *line == '>')
        continue;
    for (px = line; *px != '\n'; px++) {
        if (isupper(*px))
            *py++ = *px;
        else if (islower(*px))
            *py++ = toupper(*px);
        if (index("ATGCU",*(py-1)))
            natgc++;
    }
    *py++ = '\0';
    *py = '\0';
    (void) fclose(fp);
    dna = natgc > (llen/3);
    return(pseq+4);
}

char *
g_calloc(msg, nx, sz)                                g_calloc
{
    char *msg; /* program, calling routine */
    int nx, sz; /* number and size of elements */

    char *px; *calloc();

    if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
        if (*msg) {
            fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
            exit(1);
        }
    }
    return(px);
}

/*
 * get final jmps from dx[] or tmp file. set pp[], reset dmax: main()
 */
readjmps()
{
    int fd = -1;
    int siz, i0, i1;
    register i, j, xx;

    if (fj) {
        (void) fclose(fj);
        if ((fd = open(jname, O_RDONLY, 0)) < 0) {
            fprintf(stderr, "%s: can't open() %s\n", prog, jname);
            cleanup(1);
        }
    }
    for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; i++) {
        while (1) {
            for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)

```

Table 1 (cont')

...readjumps

```

5         if (j < 0 && dx[dmax].offset && fj) {
            (void) lseek(fd, dx[dmax].offset, 0);
            (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
            (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
            dx[dmax].ijmp = MAXJMP-1;
        }
        else
10             break;
    }
    if (i >= JMPS) {
        fprintf(stderr, "%s: too many gaps in alignment\n", prog);
        cleanup(1);
15    }
    if (j >= 0) {
        siz = dx[dmax].jp.n[j];
        xx = dx[dmax].jp.x[j];
        dmax += siz;
20        if (siz < 0) { /* gap in second seq */
            pp[1].n[i1] = -siz;
            xx += siz;
            /* id = xx - yy + len1 - 1 */
            /*
25            pp[1].x[i1] = xx - dmax + len1 - 1;
            gapy ++;
            ngapy -= siz;
            /* ignore MAXGAP when doing endgaps */
            siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
            i1 ++;
30        }
        else if (siz > 0) { /* gap in first seq */
            pp[0].n[i0] = siz;
            pp[0].x[i0] = xx;
35            gapx ++;
            ngapx += siz;
            /* ignore MAXGAP when doing endgaps */
            siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
            i0 ++;
40        }
    }
    else
        break;
}
45 /* reverse the order of jumps
*/
for (j = 0, i0--, j < i0; j++, i0--) {
    i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
    i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
50 }
for (j = 0, i1--, j < i1; j++, i1--) {
    i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
    i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
55 }
if (fd >= 0)
    (void) close(fd);
if (fj) {
    (void) unlink(jname);
    fj = 0;
60    offset = 0;
}
}

```

Table 1 (cont')

```

/*
 * write a filled jmp struct offset of the prev one (if any): nw()
 */
5  writejmps(ix)    writejmps
   int            ix;
   {
10      char        *mktemp();
      if (!fj) {
          if (mktemp(jname) < 0) {
              fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
              cleanup(1);
15          }
          if ((fj = fopen(jname, "w")) == 0) {
              fprintf(stderr, "%s: can't write %s\n", prog, jname);
              exit(1);
          }
20      }
      (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
      (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
   }
25
30
35
40
45
50
55
60

```

Table 2

PRO XXXXXXXXXXXXXXXX (Length = 15 amino acids)  
Comparison Protein XXXXXYYYYYYY (Length = 12 amino acids)

5

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-  
2) divided by (the total number of amino acid residues of the PRO polypeptide) =

10

5 divided by 15 = 33.3%

15

20

Table 3

PRO XXXXXXXXXX (Length = 10 amino acids)  
Comparison Protein XXXXXYYYYYYZZYZ (Length = 15 amino acids)

25

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-  
2) divided by (the total number of amino acid residues of the PRO polypeptide) =

30

5 divided by 10 = 50%

Table 4

5  
PRO-DNA NNNNNNNNNNNNNN (Length = 14 nucleotides)  
Comparison DNA NNNNNNLLLLLLLL (Length = 16 nucleotides)

% nucleic acid sequence identity =

10  
(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2)  
divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

15  
6 divided by 14 = 42.9%

Table 5

25  
PRO-DNA NNNNNNNNNNNN (Length = 12 nucleotides)  
Comparison DNA NNNNLLLV (Length = 9 nucleotides)

% nucleic acid sequence identity =

30  
(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2)  
divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

4 divided by 12 = 33.3%



### III. Compositions and Methods of the Invention

#### A. Full-Length PRO polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO polypeptides. In particular, cDNAs encoding various PRO polypeptides have been identified and isolated, as disclosed in further detail in the Examples below. It is noted that proteins produced in separate expression rounds may be given different PRO numbers but the UNQ number is unique for any given DNA and the encoded protein, and will not be changed. However, for sake of simplicity, in the present specification the protein encoded by the full length native nucleic acid molecules disclosed herein as well as all further native homologues and variants included in the foregoing definition of PRO, will be referred to as "PRO/number", regardless of their origin or mode of preparation.

As disclosed in the Examples below, various cDNA clones have been deposited with the ATCC. The actual nucleotide sequences of those clones can readily be determined by the skilled artisan by sequencing of the deposited clone using routine methods in the art. The predicted amino acid sequence can be determined from the nucleotide sequence using routine skill. For the PRO polypeptides and encoding nucleic acids described herein, Applicants have identified what is believed to be the reading frame best identifiable with the sequence information available at the time.

#### B. PRO Polypeptide Variants

In addition to the full-length native sequence PRO polypeptides described herein, it is contemplated that PRO variants can be prepared. PRO variants can be prepared by introducing appropriate nucleotide changes into the PRO DNA, and/or by synthesis of the desired PRO polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence PRO or in various domains of the PRO described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO that results in a change in the amino acid sequence of the PRO as compared with the native sequence PRO. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *i.e.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

PRO polypeptide fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the PRO polypeptide.

PRO fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating PRO fragments by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, PRO polypeptide fragments share at least one biological and/or immunological activity with the native PRO polypeptide disclosed herein.

10 In particular embodiments, conservative substitutions of interest are shown in Table 6 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 6, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 6

	Original Residue	Exemplary Substitutions	Preferred Substitutions
5	Ala (A)	val; leu; ile	val
	Arg (R)	lys; gln; asn	lys
	Asn (N)	gln; his; lys; arg	gln
	Asp (D)	glu	glu
	Cys (C)	ser	ser
10	Gln (Q)	asn	asn
	Glu (E)	asp	asp
	Gly (G)	pro; ala	ala
	His (H)	asn; gln; lys; arg	arg
	Ile (I)	leu; val; met; ala; phe;	
15		norleucine	leu
	Leu (L)	norleucine; ile; val;	
		met; ala; phe	ile
	Lys (K)	arg; gln; asn	arg
	Met (M)	leu; phe; ile	leu
20	Phe (F)	leu; val; ile; ala; tyr	leu
	Pro (P)	ala	ala
	Ser (S)	thr	thr
	Thr (T)	ser	ser
	Trp (W)	tyr; phe	tyr
25	Tyr (Y)	trp; phe; thr; ser	phe
	Val (V)	ile; leu; met; phe;	
		ala; norleucine	leu

30 Substantial modifications in function or immunological identity of the PRO polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- 35 (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter *et al.*, *Nucl. Acids Res.*, 13:4331 (1986); Zoller *et al.*, *Nucl. Acids Res.*, 10:6487 (1987)], cassette mutagenesis [Wells *et al.*, *Gene*, 34:315 (1985)], restriction selection mutagenesis [Wells *et al.*, *Philos. Trans. R. Soc. London SerA*, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, *Science*, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*, (W.H. Freeman & Co., N.Y.); Chothia, *J. Mol. Biol.*, 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

#### C. Modifications of PRO

Covalent modifications of PRO are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the PRO. Derivatization with bifunctional agents is useful, for instance, for crosslinking PRO to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propionimidate.

Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the  $\alpha$ -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the PRO polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence PRO (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence PRO. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to the PRO polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO (for O-linked glycosylation sites). The PRO amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the PRO polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

Removal of carbohydrate moieties present on the PRO polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, *et al.*, *Arch. Biochem. Biophys.*, 259:52 (1987) and by Edge *et al.*, *Anal. Biochem.*, 113:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.*, 138:350 (1987).

Another type of covalent modification of PRO comprises linking the PRO polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The PRO of the present invention may also be modified in a way to form a chimeric molecule comprising PRO fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the PRO with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope-tag is generally placed at the amino- or carboxyl- terminus of the PRO. The presence of such epitope-tagged forms of the PRO can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field *et al.*, *Mol. Cell. Biol.*, 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan *et al.*, *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky *et al.*, *Protein Engineering*, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp *et al.*, *BioTechnology*, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin *et al.*, *Science*, 255:192-194 (1992)]; an  $\alpha$ -tubulin epitope peptide [Skinner *et al.*, *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a

PRO polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

5 D. Preparation of PRO

The description below relates primarily to production of PRO by culturing cells transformed or transfected with a vector containing PRO nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO. For instance, the PRO sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart *et al.*,  
10 *Solid-Phase Peptide Synthesis*, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, *J. Am. Chem. Soc.*, 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO.

15 1. Isolation of DNA Encoding PRO

DNA encoding PRO may be obtained from a cDNA library prepared from tissue believed to possess the PRO mRNA and to express it at a detectable level. Accordingly, human PRO DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The PRO-encoding gene may also be obtained from a genomic library or by known synthetic procedures (e.g., automated  
20 nucleic acid synthesis).

Libraries can be screened with probes (such as antibodies to the PRO or oligonucleotides of at least about 20-30 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press,  
25 1989). An alternative means to isolate the gene encoding PRO is to use PCR methodology [Sambrook *et al.*, *supra*; Dieffenbach *et al.*, *PCR Primer: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in  
30 the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like <sup>32</sup>P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook *et al.*, *supra*.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases.  
35 Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook *et al.*, *supra*, to detect precursors and  
40 processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

## 2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in *Mammalian Cell Biotechnology: A Practical Approach*, M. Butler, ed. (IRL Press, 1991), and Sambrook *et al.*, *supra*.

Methods of eukaryotic cell transfection and prokaryotic cell transformation are known to the ordinarily skilled artisan, for example,  $\text{CaCl}_2$ ,  $\text{CaPO}_4$ , liposome-mediated and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene*, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology*, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transfections have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, *J. Bact.*, 130:946 (1977) and Hsiao *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, *Methods in Enzymology*, 185:527-537 (1990) and Mansour *et al.*, *Nature*, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as Bacilli such as *B. subtilis* and *B. licheniformis* (*e.g.*, *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan'*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan'*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No.

4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, *Nature*, 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al.*, *Bio/Technology*, 9:968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt *et al.*, *J. Bacteriol.*, 154(2):737-742 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36,906; Van den Berg *et al.*, *Bio/Technology*, 8:135 (1990)), *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna *et al.*, *J. Basic Microbiol.*, 28:265-278 [1988]); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al.*, *Proc. Natl. Acad. Sci. USA*, 76:5259-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolyocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al.*, *Biochem. Biophys. Res. Commun.*, 112:284-289 [1983]; Tilburn *et al.*, *Gene*, 26:205-221 [1983]; Yelton *et al.*, *Proc. Natl. Acad. Sci. USA*, 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, *EMBO J.*, 4:475-479 [1985]). Methylophilic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, *The Biochemistry of Methylophilic Yeasts*, 269 (1982).

Suitable host cells for the expression of glycosylated PRO are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen. Virol.*, 36:59 (1977)); Chinese hamster ovary cells/DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

### 3. Selection and Use of a Replicable Vector

The nucleic acid (e.g., cDNA or genomic DNA) encoding PRO may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.



The PRO may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 $\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb *et al.*, *Nature*, 282:39 (1979); Kingsman *et al.*, *Gene*, 7:141 (1979); Tschemper *et al.*, *Gene*, 10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, *Genetics*, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known: Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems [Chang *et al.*, *Nature*, 275:615 (1978); Goeddel *et al.*, *Nature*, 281:544 (1979)], alkaline phosphatase, a tryptophan (*trp*) promoter system [Goeddel, *Nucleic Acids Res.*, 8:4057 (1980); EP 36,776], and hybrid promoters such as the *tac* promoter [deBoer *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman *et al.*, *J. Biol. Chem.*, 255:2073 (1980)] or other glycolytic enzymes [Hess *et al.*, *J. Adv. Enzyme Reg.*, 7:149 (1968); Holland, *Biochemistry*, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase,

glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding the PRO by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO in recombinant vertebrate cell culture are described in Gething *et al.*, *Nature*, 293:620-625 (1981); Mantei *et al.*, *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

#### 4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate

directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO DNA and encoding a specific antibody epitope.

#### 5. Purification of Polypeptide

Forms of PRO may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X<sup>100</sup>) or by enzymatic cleavage. Cells employed in expression of PRO can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, *Methods in Enzymology*, 182 (1990); Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO produced.

#### E. Anti-PRO Antibodies

The present invention further provides anti-PRO antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

##### 1. Polyclonal Antibodies

The anti-PRO antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

##### 2. Monoclonal Antibodies

The anti-PRO antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the PRO polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103].

Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munsón and Pollard, *Anal. Biochem.*, 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *supra*]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the

coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison *et al.*, *supra*] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

*In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

### 3. Human and Humanized Antibodies

The anti-PRO antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin.

Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human

antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991)). Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, *Bio/Technology* 10, 779-783 (1992); Lonberg *et al.*, *Nature* 368 856-859 (1994); Morrison, *Nature* 368, 812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14, 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13 65-93 (1995).

The antibodies may also be affinity matured using known selection and/or mutagenesis methods as described above. Preferred affinity matured antibodies have an affinity which is five times, more preferably 10 times, even more preferably 20 or 30 times greater than the starting antibody (generally murine, humanized or human) from which the matured antibody is prepared.

#### 4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, *Nature*, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')<sub>2</sub> bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub> fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various technique for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V<sub>H</sub> and V<sub>L</sub> domains of one fragment are forced to pair with the complementary V<sub>L</sub> and V<sub>H</sub> domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, *J. Immunol.* 152:5368 (1994). Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, *J. Immunol.* 147:60 (1991).

Exemplary bispecific antibodies may bind to two different epitopes on a given PRO polypeptide herein. Alternatively, an anti-PRO polypeptide arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular PRO polypeptide. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular PRO polypeptide. These antibodies possess a PRO-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the PRO polypeptide and further binds tissue factor (TF).

#### 5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

#### 6. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) may be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp. Med.*, **176**: 1191-1195 (1992) and Shopes, *J. Immunol.*, **148**: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* *Cancer Research*, **53**: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, *Anti-Cancer Drug Design*, **3**: 219-230 (1989).

#### 7. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include <sup>212</sup>Bi, <sup>131</sup>I, <sup>131</sup>In, <sup>90</sup>Y, and <sup>186</sup>Re. Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional



protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) that is conjugated to a cytotoxic agent (e.g., a radionucleotide).

#### 8. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82: 3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, *J. Biol. Chem.*, 257: 286-288 (1982) via a disulfide-interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*, *J. National Cancer Inst.*, 81(19): 1484 (1989).

#### 9. Pharmaceutical Compositions of Antibodies

Antibodies specifically binding a PRO polypeptide identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of various disorders in the form of pharmaceutical compositions.

If the PRO polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, e.g., Marasco *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 7889-7893 (1993). The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules; respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

5 Such techniques are disclosed in *Remington's Pharmaceutical Sciences, supra*.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37 C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

#### F. Tissue Distribution

The location of tissues expressing the PRO can be identified by determining mRNA expression in various human tissues. The location of such genes provides information about which tissues are most likely to be affected by the stimulating and inhibiting activities of the PRO polypeptides. The location of a gene in a specific tissue also provides sample tissue for the activity blocking assays discussed below.

As noted before, gene expression in various tissues may be measured by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes.

Gene expression in various tissues, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence of a PRO polypeptide or against a synthetic peptide based on the DNA sequences encoding the PRO polypeptide or against an exogenous sequence fused to a DNA encoding a PRO polypeptide and encoding a specific antibody epitope. General techniques for generating

antibodies, and special protocols for Northern blotting and *in situ* hybridization are provided below.

G. Antibody Binding Studies

The activity of the PRO polypeptides can be further verified by antibody binding studies, in which the ability of an anti-PRO antibody to inhibit the effect of the respective PRO polypeptide on tissue cells is tested.

5 Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, the preparation of which will be described hereinbelow.

Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp. 147-158 (CRC Press, Inc., 1987).

10 Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of target protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated  
15 from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, e.g., US Pat No. 4,376,110. The second antibody may itself be  
20 labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

25 H. Cell-Based Assays

Cell-based assays and animal models for immune related diseases can be used to further understand the relationship between the genes and polypeptides identified herein and the development and pathogenesis of immune related disease.

In a different approach, cells of a cell type known to be involved in a particular immune related disease  
30 are transfected with the cDNAs described herein, and the ability of these cDNAs to stimulate or inhibit immune function is analyzed. Suitable cells can be transfected with the desired gene, and monitored for immune function activity. Such transfected cell lines can then be used to test the ability of poly- or monoclonal antibodies or antibody compositions to inhibit or stimulate immune function, for example to modulate T-cell proliferation or inflammatory cell infiltration. Cells transfected with the coding sequences of the genes  
35 identified herein can further be used to identify drug candidates for the treatment of immune related diseases.

In addition, primary cultures derived from transgenic animals (as described below) can be used in the cell-based assays herein, although stable cell lines are preferred. Techniques to derive continuous cell lines from transgenic animals are well known in the art (see, e.g., Small *et al.*, *Mol. Cell. Biol.* 5: 642-648 [1985]).

One suitable cell based assay is the mixed lymphocyte reaction (MLR). *Current Protocols in*  
40 *Immunology*, unit 3.12; edited by J E Coligan, A M Kruisbeck, D H Marglies, E M Shevach, W Strober,

National Institutes of Health. Published by John Wiley & Sons, Inc. In this assay, the ability of a test compound to stimulate or inhibit the proliferation of activated T cells is assayed. A suspension of responder T cells is cultured with allogeneic stimulator cells and the proliferation of T cells is measured by uptake of tritiated thymidine. This assay is a general measure of T cell reactivity. Since the majority of T cells respond to and produce IL-2 upon activation, differences in responsiveness in this assay in part reflect differences in IL-2 production by the responding cells. The MLR results can be verified by a standard lymphokine (IL-2) detection assay. *Current Protocols in Immunology*, above, 3.15, 6.3.

A proliferative T cell response in an MLR assay may be due to direct mitogenic properties of an assayed molecule or to external antigen induced activation. Additional verification of the T cell stimulatory activity of the PRO polypeptides can be obtained by a costimulation assay. T cell activation requires an antigen specific signal mediated through the T-cell receptor (TCR) and a costimulatory signal mediated through a second ligand binding interaction, for example, the B7 (CD80, CD86)/CD28 binding interaction. CD28 crosslinking increases lymphokine secretion by activated T cells. T cell activation has both negative and positive controls through the binding of ligands which have a negative or positive effect. CD28 and CTLA-4 are related glycoproteins in the Ig superfamily which bind to B7. CD28 binding to B7 has a positive costimulation effect of T cell activation; conversely, CTLA-4 binding to B7 has a negative T cell deactivating effect. Chambers, C. A. and Allison, J. P., *Curr. Opin. Immunol.* (1997) 9:396. Schwartz, R. H., *Cell* (1992) 71:1065; Linsey, P. S. and Ledbetter, J. A., *Annu. Rev. Immunol.* (1993) 11:191; June, C. H. *et al. Immunol. Today* (1994) 15:321; Jenkins, M. K., *Immunity* (1994) 1:405. In a costimulation assay, the PRO polypeptides are assayed for T cell costimulatory or inhibitory activity.

PRO polypeptides, as well as other compounds of the invention, which are stimulators (costimulators) of T cell proliferation and agonists, e.g., agonist antibodies, thereto as determined by MLR and costimulation assays, for example, are useful in treating immune related diseases characterized by poor, suboptimal or inadequate immune function. These diseases are treated by stimulating the proliferation and activation of T cells (and T cell mediated immunity) and enhancing the immune response in a mammal through administration of a stimulatory compound, such as the stimulating PRO polypeptides. The stimulating polypeptide may, for example, be a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940, PRO6006 polypeptide or an agonist antibody thereof.

Direct use of a stimulating compound as in the invention has been validated in experiments with 4-1BB glycoprotein, a member of the tumor necrosis factor receptor family, which binds to a ligand (4-1BBL) expressed on primed T cells and signals T cell activation and growth. Alderson, M. E. *et al., J. Immunol.* (1994) 24:2219.

The use of an agonist stimulating compound has also been validated experimentally. Activation of 4-1BB by treatment with an agonist anti-4-1BB antibody enhances eradication of tumors. Hellstrom, I. and Hellstrom, K. E., *Crit. Rev. Immunol.* (1998) 18:1. Immunoadjuvant therapy for treatment of tumors, described in more detail below, is another example of the use of the stimulating compounds of the invention.

An immune stimulating or enhancing effect can also be achieved by antagonizing or blocking the activity of a PRO which has been found to be inhibiting in the MLR assay. Negating the inhibitory activity of

the compound produces a net stimulatory effect. Suitable antagonists/blocking compounds are antibodies or fragments thereof which recognize and bind to the inhibitory protein, thereby blocking the effective interaction of the protein with its receptor and inhibiting signaling through the receptor. This effect has been validated in experiments using anti-CTLA-4 antibodies which enhance T cell proliferation, presumably by removal of the inhibitory signal caused by CTLA-4 binding. Walunas, T. L. *et al*, *Immunity* (1994) 1:405.

Alternatively, an immune stimulating or enhancing effect can also be achieved by administration of a PRO which has vascular permeability enhancing properties. Enhanced vacuolar permeability would be beneficial to disorders which can be attenuated by local infiltration of immune cells (e.g., monocytes, eosinophils, PMNs) and inflammation.

On the other hand, PRO polypeptides, as well as other compounds of the invention, which are direct inhibitors of T cell proliferation/activation, lymphokine secretion, and/or vascular permeability can be directly used to suppress the immune response. These compounds are useful to reduce the degree of the immune response and to treat immune related diseases characterized by a hyperactive, superoptimal, or autoimmune response. This use of the compounds of the invention has been validated by the experiments described above in which CTLA-4 binding to receptor B7 deactivates T cells. The direct inhibitory compounds of the invention function in an analogous manner. The use of compound which suppress vascular permeability would be expected to reduce inflammation. Such uses would be beneficial in treating conditions associated with excessive inflammation.

Alternatively, compounds, e.g., antibodies, which bind to stimulating PRO polypeptides and block the stimulating effect of these molecules produce a net inhibitory effect and can be used to suppress the T cell mediated immune response by inhibiting T cell proliferation/activation and/or lymphokine secretion. Blocking the stimulating effect of the polypeptides suppresses the immune response of the mammal. This use has been validated in experiments using an anti-IL2 antibody. In these experiments, the antibody binds to IL2 and blocks binding of IL2 to its receptor thereby achieving a T cell inhibitory effect.

#### I. Animal Models

The results of the cell based *in vitro* assays can be further verified using *in vivo* animal models and assays for T-cell function. A variety of well known animal models can be used to further understand the role of the genes identified herein in the development and pathogenesis of immune related disease, and to test the efficacy of candidate therapeutic agents, including antibodies, and other antagonists of the native polypeptides, including small molecule antagonists. The *in vivo* nature of such models makes them predictive of responses in human patients. Animal models of immune related diseases include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, e.g., murine models. Such models can be generated by introducing cells into syngeneic mice using standard techniques, e.g., subcutaneous injection, tail vein injection, spleen implantation, intraperitoneal implantation, implantation under the renal capsule, etc.

Graft-versus-host disease occurs when immunocompetent cells are transplanted into immunosuppressed or tolerant patients. The donor cells recognize and respond to host antigens. The response can vary from life threatening severe inflammation to mild cases of diarrhea and weight loss. Graft-versus-host disease models provide a means of assessing T cell reactivity against MHC antigens and minor transplant antigens. A suitable procedure is described in detail in Current Protocols in Immunology, above, unit 4.3.

An animal model for skin allograft rejection is a means of testing the ability of T cells to mediate *in vivo* tissue destruction and a measure of their role in transplant rejection. The most common and accepted models use murine tail-skin grafts. Repeated experiments have shown that skin allograft rejection is mediated by T cells, helper T cells and killer-effector T cells, and not antibodies. Auchincloss, H. Jr. and Sachs, D. H.,  
5 *Fundamental Immunology*, 2nd ed., W. E. Paul ed., Raven Press, NY, 1989, 889-992. A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.4. Other transplant rejection models which can be used to test the compounds of the invention are the allogeneic heart transplant models described by Tanabe, M. *et al*, *Transplantation* (1994) 58:23 and Tinubu, S. A. *et al*, *J. Immunol.* (1994) 4330-4338.

Animal models for delayed type hypersensitivity provides an assay of cell mediated immune function  
10 as well. Delayed type hypersensitivity reactions are a T cell mediated *in vivo* immune response characterized by inflammation which does not reach a peak until after a period of time has elapsed after challenge with an antigen. These reactions also occur in tissue specific autoimmune diseases such as multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE, a model for MS). A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.5.

15 EAE is a T cell mediated autoimmune disease characterized by T cell and mononuclear cell inflammation and subsequent demyelination of axons in the central nervous system. EAE is generally considered to be a relevant animal model for MS in humans. Bolton, C., *Multiple Sclerosis* (1995) 1:143. Both acute and relapsing-remitting models have been developed. The compounds of the invention can be tested for T cell stimulatory or inhibitory activity against immune mediated demyelinating disease using the protocol  
20 described in *Current Protocols in Immunology*, above, units 15.1 and 15.2. See also the models for myelin disease in which oligodendrocytes or Schwann cells are grafted into the central nervous system as described in Duncan, I. D. *et al*, *Molec. Med. Today* (1997) 554-561.

Contact hypersensitivity is a simple delayed type hypersensitivity *in vivo* assay of cell mediated immune function. In this procedure, cutaneous exposure to exogenous haptens which gives rise to a delayed  
25 type hypersensitivity reaction which is measured and quantitated. Contact sensitivity involves an initial sensitizing phase followed by an elicitation phase. The elicitation phase occurs when the T lymphocytes encounter an antigen to which they have had previous contact. Swelling and inflammation occur, making this an excellent model of human allergic contact dermatitis. A suitable procedure is described in detail in *Current Protocols in Immunology*, Eds. J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W.  
30 Strober, John Wiley & Sons, Inc., 1994, unit 4.2. See also Grabbe, S. and Schwarz, T., *Immun. Today* 19 (1): 37-44 (1998).

An animal model for arthritis is collagen-induced arthritis. This model shares clinical, histological and immunological characteristics of human autoimmune rheumatoid arthritis and is an acceptable model for human autoimmune arthritis. Mouse and rat models are characterized by synovitis, erosion of cartilage and  
35 subchondral bone. The compounds of the invention can be tested for activity against autoimmune arthritis using the protocols described in *Current Protocols in Immunology*, above, units 15.5. See also the model using a monoclonal antibody to CD18 and VLA-4 integrins described in Issekutz, A.C. *et al*, *Immunology* (1996) 88:569.

A model of asthma has been described in which antigen-induced airway hyper-reactivity, pulmonary  
40 eosinophilia and inflammation are induced by sensitizing an animal with ovalbumin and then challenging the

animal with the same protein delivered by aerosol. Several animal models (guinea pig, rat, non-human primate) show symptoms similar to atopic asthma in humans upon challenge with aerosol antigens. Murine models have many of the features of human asthma. Suitable procedures to test the compounds of the invention for activity and effectiveness in the treatment of asthma are described by Wolyniec, W. W. *et al. Am. J. Respir. Cell Mol.*

5 *Biol.* (1998) 18:777 and the references cited therein.

Additionally, the compounds of the invention can be tested on animal models for psoriasis like diseases. Evidence suggests a T cell pathogenesis for psoriasis. The compounds of the invention can be tested in the scid/scid mouse model described by Schon, M. P. *et al. Nat. Med.* (1997) 3:183, in which the mice demonstrate histopathologic skin lesions resembling psoriasis. Another suitable model is the human skin/scid mouse chimera prepared as described by Nickoloff, B. J. *et al. Am. J. Path.* (1995) 146:580.

10 Recombinant (transgenic) animal models can be engineered by introducing the coding portion of the genes identified herein into the genome of animals of interest, using standard techniques for producing transgenic animals. Animals that can serve as a target for transgenic manipulation include, without limitation, mice, rats, rabbits, guinea pigs, sheep, goats, pigs, and non-human primates, e.g., baboons, chimpanzees and monkeys. Techniques known in the art to introduce a transgene into such animals include pronucleic microinjection (Hoppe and Wanger, U.S. Patent No. 4,873,191); retrovirus-mediated gene transfer into germ lines (e.g., Van der Putten *et al., Proc. Natl. Acad. Sci. USA* 82, 6148-615 [1985]); gene targeting in embryonic stem cells (Thompson *et al., Cell* 56, 313-321 [1989]); electroporation of embryos (Lo, *Mol. Cell. Biol.* 3, 1803-1814 [1983]); sperm-mediated gene transfer (Lavitrano *et al., Cell* 57, 717-73 [1989]). For review, see, for example, U.S. Patent No. 4,736,866.

20 For the purpose of the present invention, transgenic animals include those that carry the transgene only in part of their cells ("mosaic animals"). The transgene can be integrated either as a single transgene, or in concatamers, e.g., head-to-head or head-to-tail tandems. Selective introduction of a transgene into a particular cell type is also possible by following, for example, the technique of Lasko *et al., Proc. Natl. Acad. Sci. USA* 89, 6232-636 (1992).

25 The expression of the transgene in transgenic animals can be monitored by standard techniques. For example, Southern blot analysis or PCR amplification can be used to verify the integration of the transgene. The level of mRNA expression can then be analyzed using techniques such as *in situ* hybridization, Northern blot analysis, PCR, or immunocytochemistry.

30 The animals may be further examined for signs of immune disease pathology, for example by histological examination to determine infiltration of immune cells into specific tissues. Blocking experiments can also be performed in which the transgenic animals are treated with the compounds of the invention to determine the extent of the T cell proliferation stimulation or inhibition of the compounds. In these experiments, blocking antibodies which bind to the PRO polypeptide, prepared as described above, are administered to the animal and the effect on immune function is determined.

35 Alternatively, "knock out" animals can be constructed which have a defective or altered gene encoding a polypeptide identified herein, as a result of homologous recombination between the endogenous gene encoding the polypeptide and altered genomic DNA encoding the same polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding a particular polypeptide can be used to clone genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic

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DNA encoding a particular polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li et al., *Cell*, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the polypeptide.

15 J. ImmunoAdjuvant Therapy

In one embodiment, the immunostimulating compounds of the invention can be used in immunoadjuvant therapy for the treatment of tumors (cancer). It is now well established that T cells recognize human tumor specific antigens. One group of tumor antigens, encoded by the MAGE, BAGE and GAGE families of genes, are silent in all adult normal tissues, but are expressed in significant amounts in tumors, such as melanomas, lung tumors, head and neck tumors, and bladder carcinomas. DeSmet, C. et al., (1996) *Proc. Natl. Acad. Sci. USA*, 93:7149. It has been shown that costimulation of T cells induces tumor regression and an antitumor response both *in vitro* and *in vivo*. Melero, I. et al., *Nature Medicine* (1997) 3:682; Kwon, E. D. et al., *Proc. Natl. Acad. Sci. USA* (1997) 94: 8099; Lynch, D. H. et al, *Nature Medicine* (1997) 3:625; Finn, O. J. and Lotze, M. T., *J. Immunol.* (1998) 21:114. The stimulatory compounds of the invention can be administered as adjuvants, alone or together with a growth regulating agent, cytotoxic agent or chemotherapeutic agent, to stimulate T cell proliferation/activation and an antitumor response to tumor antigens. The growth regulating, cytotoxic, or chemotherapeutic agent may be administered in conventional amounts using known administration regimes. Immunostimulating activity by the compounds of the invention allows reduced amounts of the growth regulating, cytotoxic, or chemotherapeutic agents thereby potentially lowering the toxicity to the patient.

30 K. Screening Assays for Drug Candidates

Screening assays for drug candidates are designed to identify compounds that bind to or complex with the polypeptides encoded by the genes identified herein or a biologically active fragment thereof, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds, including peptides, preferably soluble peptides, (poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays,



immunoassays and cell based assays, which are well characterized in the art.

All assays are common in that they call for contacting the drug candidate with a polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

5 In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, e.g., on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the polypeptide and drying. Alternatively, an immobilized antibody, e.g., a monoclonal antibody, specific for the  
10 polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, e.g., the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, e.g., by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the  
15 surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labelled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular protein encoded by a gene identified herein, its interaction with that protein can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, *Nature (London)* 340, 245-246 (1989); Chien *et al.*, *Proc. Natl. Acad. Sci. USA* 88, 9578-9582 (1991)] as disclosed by Chevray and Nathans, *Proc. Natl. Acad. Sci. USA* 89, 5789-5793 (1991). Many  
25 transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, while the other one functioning as the transcription activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on  
30 reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for  $\beta$ -galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein  
35 interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

In order to find compounds that interfere with the interaction of a gene identified herein and other intra- or extracellular components can be tested, a reaction mixture is usually prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a test compound to inhibit binding, the reaction is run in the  
40 absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction

mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described above. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

5 L. Compositions and Methods for the Treatment of Immune Related Diseases

The compositions useful in the treatment of immune related diseases include, without limitation, proteins, antibodies, small organic molecules, peptides, phosphopeptides, antisense and ribozyme molecules, triple helix molecules, *etc.* that inhibit or stimulate immune function, for example, T cell proliferation/activation, lymphokine release, or immune cell infiltration.

10 For example, antisense RNA and RNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation initiation site, *e.g.*, between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA.  
15 Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, *e.g.*, Rossi, *Current Biology* 4, 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple helix formation used to inhibit transcription should be single-stranded  
20 and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, *e.g.*, PCT publication No. WO 97/33551, *supra*.

These molecules can be identified by any or any combination of the screening assays discussed above  
25 and/or by any other screening techniques well known for those skilled in the art.

M. Pharmaceutical Compositions

The active PRO molecules of the invention (*e.g.*, PRO polypeptides, anti-PRO antibodies, and/or variants of each) as well as other molecules identified by the screening assays disclosed above, can be administered for the treatment of immune related diseases, in the form of pharmaceutical compositions.

30 Therapeutic formulations of the active PRO molecule, preferably a polypeptide or antibody of the invention, are prepared for storage by mixing the active molecule having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences*, 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and  
35 include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic  
40 polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine,

or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counterions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN<sup>TM</sup>, PLURONICS<sup>TM</sup> or polyethylene glycol (PEG).

5       Compounds identified by the screening assays disclosed herein can be formulated in an analogous manner, using standard techniques well known in the art.

Lipofections or liposomes can also be used to deliver the PRO molecule into cells. Where antibody fragments are used, the smallest inhibitory fragment which specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable region sequences of an antibody, peptide molecules  
10       can be designed which retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology (see, e.g., Marasco *et al.*, *Proc. Natl. Acad. Sci. USA* 90, 7889-7893 [1993]).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect  
15       each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active PRO molecules may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-  
20       microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by  
25       filtration through sterile filtration membranes.

Sustained-release preparations of the PRO molecules may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or  
30       poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$ -ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time  
35       periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic  
40       solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix

compositions.

N. Methods of Treatment

It is contemplated that the polypeptides, antibodies and other active compounds of the present invention may be used to treat various immune related diseases and conditions, such as T cell mediated diseases, including those characterized by infiltration of inflammatory cells into a tissue, stimulation of T-cell proliferation, inhibition of T-cell proliferation, increased or decreased vascular permeability or the inhibition thereof.

Exemplary conditions or disorders to be treated with the polypeptides, antibodies and other compounds of the invention, include, but are not limited to systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, osteoarthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft-versus-host-disease.

In systemic lupus erythematosus, the central mediator of disease is the production of auto-reactive antibodies to self proteins/tissues and the subsequent generation of immune-mediated inflammation. antibodies either directly or indirectly mediate tissue injury. Though T lymphocytes have not been shown to be directly involved in tissue damage, T lymphocytes are required for the development of auto-reactive antibodies. The genesis of the disease is thus T lymphocyte dependent. Multiple organs and systems are affected clinically including kidney, lung, musculoskeletal system, mucocutaneous, eye, central nervous system, cardiovascular system, gastrointestinal tract, bone marrow and blood.

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that mainly involves the synovial membrane of multiple joints with resultant injury to the articular cartilage. The pathogenesis is T lymphocyte dependent and is associated with the production of rheumatoid factors, auto-antibodies directed against self IgG, with the resultant formation of immune complexes that attain high levels in joint fluid and blood. These complexes in the joint may induce the marked infiltrate of lymphocytes and monocytes into the synovium and subsequent marked synovial changes; the joint space/fluid if infiltrated by similar cells with the addition of numerous neutrophils. Tissues affected are primarily the joints, often in symmetrical pattern. However, extra-articular disease also occurs in two major forms. One form is the

development of extra-articular lesions with ongoing progressive joint disease and typical lesions of pulmonary fibrosis, vasculitis, and cutaneous ulcers. The second form of extra-articular disease is the so called Felty's syndrome which occurs late in the RA disease course, sometimes after joint disease has become quiescent, and involves the presence of neutropenia, thrombocytopenia and splenomegaly. This can be accompanied by vasculitis in multiple organs with formations of infarcts, skin ulcers and gangrene. Patients often also develop rheumatoid nodules in the subcutis tissue overlying affected joints; the nodules late stage have necrotic centers surrounded by a mixed inflammatory cell infiltrate. Other manifestations which can occur in RA include: pericarditis, pleuritis, coronary arteritis, interstitial pneumonitis with pulmonary fibrosis, keratoconjunctivitis sicca, and rheumatoid nodules.

Juvenile chronic arthritis is a chronic idiopathic inflammatory disease which begins often at less than 16 years of age. Its phenotype has some similarities to RA; some patients which are rheumatoid factor positive are classified as juvenile rheumatoid arthritis. The disease is sub-classified into three major categories: pauciarticular, polyarticular, and systemic. The arthritis can be severe and is typically destructive and leads to joint ankylosis and retarded growth. Other manifestations can include chronic anterior uveitis and systemic amyloidosis.

Spondyloarthropathies are a group of disorders with some common clinical features and the common association with the expression of HLA-B27 gene product. The disorders include: ankylosing spondylitis, Reiter's syndrome (reactive arthritis), arthritis associated with inflammatory bowel disease, spondylitis associated with psoriasis, juvenile onset spondyloarthropathy and undifferentiated spondyloarthropathy. Distinguishing features include sacroileitis with or without spondylitis; inflammatory asymmetric arthritis; association with HLA-B27 (a serologically defined allele of the HLA-B locus of class I MHC); ocular inflammation, and absence of autoantibodies associated with other rheumatoid disease. The cell most implicated as key to induction of the disease is the CD8+ T lymphocyte, a cell which targets antigen presented by class I MHC molecules. CD8+ T cells may react against the class I MHC allele HLA-B27 as if it were a foreign peptide expressed by MHC class I molecules. It has been hypothesized that an epitope of HLA-B27 may mimic a bacterial or other microbial antigenic epitope and thus induce a CD8+ T cells response.

Systemic sclerosis (scleroderma) has an unknown etiology. A hallmark of the disease is induration of the skin; likely this is induced by an active inflammatory process. Scleroderma can be localized or systemic; vascular lesions are common and endothelial cell injury in the microvasculature is an early and important event in the development of systemic sclerosis; the vascular injury may be immune mediated. An immunologic basis is implied by the presence of mononuclear cell infiltrates in the cutaneous lesions and the presence of anti-nuclear antibodies in many patients. ICAM-1 is often upregulated on the cell surface of fibroblasts in skin lesions suggesting that T cell interaction with these cells may have a role in the pathogenesis of the disease. Other organs involved include: the gastrointestinal tract: smooth muscle atrophy and fibrosis resulting in abnormal peristalsis/motility; kidney: concentric subendothelial intimal proliferation affecting small arcuate and interlobular arteries with resultant reduced renal cortical blood flow, results in proteinuria, azotemia and hypertension; skeletal muscle: atrophy, interstitial fibrosis; inflammation; lung: interstitial pneumonitis and interstitial fibrosis; and heart: contraction band necrosis, scarring/fibrosis.

Idiopathic inflammatory myopathies including dermatomyositis, polymyositis and others are disorders of chronic muscle inflammation of unknown etiology resulting in muscle weakness. Muscle

injury/inflammation is often symmetric and progressive. Autoantibodies are associated with most forms. These myositis-specific autoantibodies are directed against and inhibit the function of components, proteins and RNA's, involved in protein synthesis.

Sjögren's syndrome is due to immune-mediated inflammation and subsequent functional destruction of the tear glands and salivary glands. The disease can be associated with or accompanied by inflammatory connective tissue diseases. The disease is associated with autoantibody production against Ro and La antigens, both of which are small RNA-protein complexes. Lesions result in keratoconjunctivitis sicca, xerostomia, with other manifestations or associations including biliary cirrhosis, peripheral or sensory neuropathy, and palpable purpura.

Systemic vasculitis are diseases in which the primary lesion is inflammation and subsequent damage to blood vessels which results in ischemia/necrosis/degeneration to tissues supplied by the affected vessels and eventual end-organ dysfunction in some cases. Vasculitides can also occur as a secondary lesion or sequelae to other immune-inflammatory mediated diseases such as rheumatoid arthritis, systemic sclerosis, *etc.*, particularly in diseases also associated with the formation of immune complexes. Diseases in the primary systemic vasculitis group include: systemic necrotizing vasculitis; polyarteritis nodosa, allergic angiitis and granulomatosis, polyangiitis; Wegener's granulomatosis; lymphomatoid granulomatosis; and giant cell arteritis. Miscellaneous vasculitides include: mucocutaneous lymph node syndrome (MLNS or Kawasaki's disease), isolated CNS vasculitis, Behr's disease, thromboangiitis obliterans (Buerger's disease) and cutaneous necrotizing venulitis. The pathogenic mechanism of most of the types of vasculitis listed is believed to be primarily due to the deposition of immunoglobulin complexes in the vessel wall and subsequent induction of an inflammatory response either via ADCC, complement activation, or both.

Sarcoidosis is a condition of unknown etiology which is characterized by the presence of epithelioid granulomas in nearly any tissue in the body; involvement of the lung is most common. The pathogenesis involves the persistence of activated macrophages and lymphoid cells at sites of the disease with subsequent chronic sequelae resultant from the release of locally and systemically active products released by these cell types.

Autoimmune hemolytic anemia including autoimmune hemolytic anemia, immune pancytopenia, and paroxysmal nocturnal hemoglobinuria is a result of production of antibodies that react with antigens expressed on the surface of red blood cells (and in some cases other blood cells including platelets as well) and is a reflection of the removal of those antibody coated cells via complement mediated lysis and/or ADCC/Fc-receptor-mediated mechanisms.

In autoimmune thrombocytopenia including thrombocytopenic purpura, and immune-mediated thrombocytopenia in other clinical settings, platelet destruction/removal occurs as a result of either antibody or complement attaching to platelets and subsequent removal by complement lysis, ADCC or FC-receptor mediated mechanisms.

Thyroiditis including Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, and atrophic thyroiditis, are the result of an autoimmune response against thyroid antigens with production of antibodies that react with proteins present in and often specific for the thyroid gland. Experimental models exist including spontaneous models: rats (BUF and BB rats) and chickens (obese chicken strain); inducible models: immunization of animals with either thyroglobulin, thyroid microsomal antigen (thyroid peroxidase).

Type I diabetes mellitus or insulin-dependent diabetes is the autoimmune destruction of pancreatic islet  $\beta$  cells; this destruction is mediated by auto-antibodies and auto-reactive T cells. Antibodies to insulin or the insulin receptor can also produce the phenotype of insulin-non-responsiveness.

Immune mediated renal diseases, including glomerulonephritis and tubulointerstitial nephritis, are the result of antibody or T lymphocyte mediated injury to renal tissue either directly as a result of the production of autoreactive antibodies or T cells against renal antigens or indirectly as a result of the deposition of antibodies and/or immune complexes in the kidney that are reactive against other, non-renal antigens. Thus other immune-mediated diseases that result in the formation of immune-complexes can also induce immune mediated renal disease as an indirect sequelae. Both direct and indirect immune mechanisms result in inflammatory response that produces/induces lesion development in renal tissues with resultant organ function impairment and in some cases progression to renal failure. Both humoral and cellular immune mechanisms can be involved in the pathogenesis of lesions.

Demyelinating diseases of the central and peripheral nervous systems, including Multiple Sclerosis; idiopathic demyelinating polyneuropathy or Guillain-Barre syndrome; and Chronic Inflammatory Demyelinating Polyneuropathy, are believed to have an autoimmune basis and result in nerve demyelination as a result of damage caused to oligodendrocytes or to myelin directly. In MS there is evidence to suggest that disease induction and progression is dependent on T lymphocytes. Multiple Sclerosis is a demyelinating disease that is T lymphocyte-dependent and has either a relapsing-remitting course or a chronic progressive course. The etiology is unknown; however, viral infections, genetic predisposition, environment, and autoimmunity all contribute. Lesions contain infiltrates of predominantly T lymphocyte mediated, microglial cells and infiltrating macrophages; CD4+T lymphocytes are the predominant cell type at lesions. The mechanism of oligodendrocyte cell death and subsequent demyelination is not known but is likely T lymphocyte driven.

Inflammatory and Fibrotic Lung Disease, including Eosinophilic Pneumonias; Idiopathic Pulmonary Fibrosis, and Hypersensitivity Pneumonitis may involve a dysregulated immune-inflammatory response. Inhibition of that response would be of therapeutic benefit.

Autoimmune or Immune-mediated Skin Disease including Bullous Skin Diseases, Erythema Multiforme, and Contact Dermatitis are mediated by auto-antibodies, the genesis of which is T lymphocyte-dependent.

Psoriasis is a T lymphocyte-mediated inflammatory disease. Lesions contain infiltrates of T lymphocytes, macrophages and antigen processing cells, and some neutrophils.

Allergic diseases, including asthma; allergic rhinitis; atopic dermatitis; food hypersensitivity; and urticaria are T lymphocyte dependent. These diseases are predominantly mediated by T lymphocyte induced inflammation, IgE mediated-inflammation or a combination of both.

Transplantation associated diseases, including Graft rejection and Graft-Versus-Host-Disease (GVHD) are T lymphocyte-dependent; inhibition of T lymphocyte function is ameliorative.

Other diseases in which intervention of the immune and/or inflammatory response have benefit are infectious disease including but not limited to viral infection (including but not limited to AIDS, hepatitis A, B, C, D, E and herpes) bacterial infection, fungal infections, and protozoal and parasitic infections (molecules (or derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response to infectious agents), diseases of immunodeficiency (molecules/derivatives/agonists) which stimulate the MLR

can be utilized therapeutically to enhance the immune response for conditions of inherited, acquired, infectious induced (as in HIV infection), or iatrogenic (*i.e.*, as from chemotherapy) immunodeficiency), and neoplasia.

It has been demonstrated that some human cancer patients develop an antibody and/or T lymphocyte response to antigens on neoplastic cells. It has also been shown in animal models of neoplasia that enhancement of the immune response can result in rejection or regression of that particular neoplasm. Molecules that enhance the T lymphocyte response in the MLR have utility *in vivo* in enhancing the immune response against neoplasia. Molecules which enhance the T lymphocyte proliferative response in the MLR (or small molecule agonists or antibodies that affected the same receptor in an agonistic fashion) can be used therapeutically to treat cancer. Molecules that inhibit the lymphocyte response in the MLR also function *in vivo* during neoplasia to suppress the immune response to a neoplasm; such molecules can either be expressed by the neoplastic cells themselves or their expression can be induced by the neoplasm in other cells. Antagonism of such inhibitory molecules (either with antibody, small molecule antagonists or other means) enhances immune-mediated tumor rejection.

Additionally, inhibition of molecules with proinflammatory properties may have therapeutic benefit in reperfusion injury; stroke; myocardial infarction; atherosclerosis; acute lung injury; hemorrhagic shock; burn; sepsis/septic shock; acute tubular necrosis; endometriosis; degenerative joint disease and pancreatitis.

The compounds of the present invention, *e.g.*, polypeptides or antibodies, are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation (intranasal, intrapulmonary) routes. Intravenous or inhaled administration of polypeptides and antibodies is preferred.

In immunoadjuvant therapy, other therapeutic regimens, such administration of an anti-cancer agent, may be combined with the administration of the proteins, antibodies or compounds of the instant invention. For example, the patient to be treated with a the immunoadjuvant of the invention may also receive an anti-cancer agent (chemotherapeutic agent) or radiation therapy. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of the immunoadjuvant or may be given simultaneously therewith. Additionally, an anti-oestrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) may be given in dosages known for such molecules.

It may be desirable to also administer antibodies against other immune disease associated or tumor associated antigens, such as antibodies which bind to CD20, CD11a, CD18, ErbB2, EGFR, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be coadministered to the patient. Sometimes, it may be beneficial to also administer one or more cytokines to the patient. In one embodiment, the PRO polypeptides are coadministered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by a PRO polypeptide. However, simultaneous administration or administration first is also contemplated. Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and the PRO polypeptide.

For the treatment or reduction in the severity of immune related disease, the appropriate dosage of an a



compound of the invention will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the compound, and the discretion of the attending physician. The compound is suitably administered to the patient at one time or over a series of treatments.

- 5 For example, depending on the type and severity of the disease, about 1  $\mu\text{g/kg}$  to 15  $\text{mg/kg}$  (e.g., 0.1-20  $\text{mg/kg}$ ) of polypeptide or antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1  $\mu\text{g/kg}$  to 100  $\text{mg/kg}$  or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a  
10 desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

O. Articles of Manufacture

- In another embodiment of the invention, an article of manufacture containing materials (e.g., comprising a PRO molecule) useful for the diagnosis or treatment of the disorders described above is provided.  
15 The article of manufacture comprises a container and an instruction. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is usually a  
20 polypeptide or an antibody of the invention. An instruction or label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and  
25 package inserts with instructions for use.

P. Diagnosis and Prognosis of Immune Related Disease

- Cell surface proteins, such as proteins which are overexpressed in certain immune related diseases, are excellent targets for drug candidates or disease treatment. The same proteins along with secreted proteins encoded by the genes amplified in immune related disease states find additional use in the diagnosis and  
30 prognosis of these diseases. For example, antibodies directed against the protein products of genes amplified in multiple sclerosis, rheumatoid arthritis, or another immune related disease, can be used as diagnostics or prognostics.

- For example, antibodies, including antibody fragments, can be used to qualitatively or quantitatively detect the expression of proteins encoded by amplified or overexpressed genes ("marker gene products"). The  
35 antibody preferably is equipped with a detectable, e.g., fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. These techniques are particularly suitable, if the overexpressed gene encodes a cell surface protein. Such binding assays are performed essentially as described above.

- In situ* detection of antibody binding to the marker gene products can be performed, for example, by  
40 immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed

from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for *in situ* detection.

5 The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

#### 10 EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA. Unless otherwise noted, the present invention uses standard procedures of recombinant DNA technology, such as those described hereinabove and in the following textbooks: Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press N.Y., 1989; Ausubel *et al.*, *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y., 1989; Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications*, Academic Press, inc., N.Y., 1990; Harlow *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, 1988; Gait, M.J., *Oligonucleotide Synthesis*, IRL Press, Oxford, 1984; R.I. Freshney, *Animal Cell Culture*, 1987; Coligan *et al.*, *Current Protocols in Immunology*, 1991.

#### EXAMPLE 1

Isolation of cDNA clones Encoding Human PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940, PRO6006 polypeptide.

Various techniques were employed for isolating the cDNA clones described below. A general description of the methods employed follows immediately hereafter, whereas the details relating the specific sequences isolated is recited separately for each native sequence. It is understood that the actual sequences of the PRO polypeptides are those which are contained within or encoded by the clone deposited with the ATCC - and that in the in event of any discrepancy between the sequence deposited and the sequence disclosed herein, the sequence of the deposit is the true sequence

#### ECD Homology:

35 The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public EST databases (e.g., GenBank), a private EST database (LIFESEQ<sup>®</sup>, Incyte Pharmaceuticals, Palo Alto, CA), and proprietary ESTs from Genentech. The search was performed using the computer program BLAST or BLAST2 [Altschul *et al.*, *Methods in Enzymology*, 266: 460-480 (1996)] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons

resulting in a BLAST score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

Using various ESTs, drawing from both public and private databases, a consensus DNA sequence was assembled. Oligonucleotides were then synthesized to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone encoding the particular native sequence PRO polypeptide identified herein.

In order to screen several libraries for a source of a full-length, native sequence clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified below. A positive library was then used to isolate clones encoding the particular native sequence PRO polypeptide using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from various human tissue libraries, including, *e.g.*, fetal lung, fetal liver, fetal brain, small intestine, smooth muscle cells, *etc.* The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB; pRKB is a precursor of pRK5D that does not contain the SfiI site; see, Holmes *et al.*, *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites. The clones were sequenced using known and readily available methodology.

*Amylase yeast screen:*

1. Preparation of oligo dT primed cDNA library

mRNA was isolated from various tissues (*e.g.*, such as those indicated above under the ECD homology procedure) using reagents and protocols from Invitrogen, San Diego, CA (Fast Track 2). This RNA was used to generate an oligo dT primed cDNA library in the vector pRK5D using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System). In this procedure, the double stranded cDNA was sized to greater than 1000 bp and the SalI/NotI linked cDNA was cloned into XhoI/NotI cleaved vector. pRK5D is a cloning vector that has an sp6 transcription initiation site followed by an SfiI restriction enzyme site preceding the XhoI/NotI cDNA cloning sites.

2. Preparation of random primed cDNA library

A secondary cDNA library was generated in order to preferentially represent the 5' ends of the primary cDNA clones. Sp6 RNA was generated from the primary library (described above), and this RNA was used to generate a random primed cDNA library in the vector pSST-AMY.0 using reagents and protocols from Life Technologies (Super Script Plasmid System, referenced above). In this procedure the double stranded cDNA was sized to 500-1000 bp, linked with blunt to NotI adaptors, cleaved with SfiI, and cloned into SfiI/NotI cleaved vector. pSST-AMY.0 is a cloning vector that has a yeast alcohol dehydrogenase promoter preceding the cDNA cloning sites and the mouse amylase sequence (the mature sequence without the secretion signal) followed by the yeast alcohol dehydrogenase terminator, after the cloning sites. Thus, cDNAs cloned into this vector that are fused in frame with amylase sequence will lead to the secretion of amylase from appropriately transfected yeast colonies.

3. Transformation and Detection

DNA from the library described in paragraph 2 above was chilled on ice to which was added electrocompetent DH10B bacteria (Life Technologies, 20 ml). The bacteria and vector mixture was then electroporated as recommended by the manufacturer. Subsequently, SOC media (Life Technologies, 1 ml) was added and the mixture was incubated at 37°C for 30 minutes. The transformants were then plated onto 20 standard 150 mm LB plates containing ampicillin and incubated for 16 hours (37°C). Positive colonies were scraped off the plates and the DNA was isolated from the bacterial pellet using standard protocols, e.g., Ciscogradient. The purified DNA was then carried on to the yeast protocols below.

The yeast methods were divided into three categories: (1) Transformation of yeast with the plasmid/cDNA combined vector; (2) Detection and isolation of yeast clones secreting amylase; and (3) PCR amplification of the insert directly from the yeast colony and purification of the DNA for sequencing and further analysis.

The yeast strain used was HD56-5A (ATCC-90785). This strain has the following genotype: MAT<sup>+</sup> alpha, ura3-52, leu2-3, leu2-112, his3-11, his3-15, MAL<sup>+</sup>, SUC<sup>+</sup>, GAL<sup>+</sup>. Preferably, yeast mutants can be employed that have deficient post-translational pathways. Such mutants may have translocation deficient alleles in *sec71*, *sec72*, *sec62*, with truncated *sec71* being most preferred. Alternatively, antagonists (including antisense nucleotides and/or ligands) which interfere with the normal operation of these genes, other proteins implicated in this post translation pathway (e.g., SEC61p, SEC72p, SEC62p, SEC63p, TDI1p or SSA1p-4p) or the complex formation of these proteins may also be preferably employed in combination with the amylase-expressing yeast.

Transformation was performed based on the protocol outlined by Gietz *et al.*, *Nucl. Acid. Res.*, 20:1425 (1992). Transformed cells were then inoculated from agar into YEPD complex media broth (100 ml) and grown overnight at 30°C. The YEPD broth was prepared as described in Kaiser *et al.*, *Methods in Yeast Genetics*, Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 207 (1994). The overnight culture was then diluted to about  $2 \times 10^6$  cells/ml (approx. OD<sub>600</sub> = 0.1) into fresh YEPD broth (500 ml) and regrown to  $1 \times 10^7$  cells/ml (approx. OD<sub>600</sub>=0.4-0.5).

The cells were then harvested and prepared for transformation by transfer into GS3 rotor bottles in a Sorval GS3 rotor at 5,000 rpm for 5 minutes, the supernatant discarded, and then resuspended into sterile water, and centrifuged again in 50 ml falcon tubes at 3,500 rpm in a Beckman GS-6KR centrifuge. The supernatant was discarded and the cells were subsequently washed with LiAc/TE (10 ml, 10 mM Tris-HCl, 1 mM EDTA pH 7.5, 100 mM Li2OCCCH3), and resuspended into LiAc/TE (2.5 ml).

Transformation took place by mixing the prepared cells (100 µl) with freshly denatured single stranded salmon testes DNA (Lofstrand Labs, Gaithersburg, MD) and transforming DNA (1 µg, vol. < 10 µl) in microfuge tubes. The mixture was mixed briefly by vortexing, then 40% PEG/TE (600 µl, 40% polyethylene glycol-4000, 10 mM Tris-HCl, 1 mM EDTA, 100 mM Li2Ac, pH 7.5) was added. This mixture was gently mixed and incubated at 30°C while agitating for 30 minutes. The cells were then heat shocked at 42°C for 15 minutes, and the reaction vessel centrifuged in a microfuge at 12,000 rpm for 5-10 seconds, decanted and resuspended into TE (500 µl, 10 mM Tris-HCl, 1 mM EDTA pH 7.5) followed by recentrifugation. The cells were then diluted into TE (1 ml) and aliquots (200 µl) were spread onto the selective media previously prepared in 150 mm growth plates (VWR).

Alternatively, instead of multiple small reactions, the transformation was performed using a single, large scale reaction, wherein reagent amounts were scaled up accordingly.

The selective media used was a synthetic complete dextrose agar lacking uracil (SCD-Ura) prepared as described in Kaiser *et al.*, *Methods in Yeast Genetics*, Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 208-210 (1994). Transformants were grown at 30°C for 2-3 days.

The detection of colonies secreting amylase was performed by including red starch in the selective growth media. Starch was coupled to the red dye (Reactive Red-120, Sigma) as per the procedure described by Biely *et al.*, *Anal. Biochem.*, 172:176-179 (1988). The coupled starch was incorporated into the SCD-Ura agar plates at a final concentration of 0.15% (w/v), and was buffered with potassium phosphate to a pH of 7.0 (50-100 mM final concentration).

The positive colonies were picked and streaked across fresh selective media (onto 150 mm plates) in order to obtain well isolated and identifiable single colonies. Well isolated single colonies positive for amylase secretion were detected by direct incorporation of red starch into buffered SCD-Ura agar. Positive colonies were determined by their ability to break down starch resulting in a clear halo around the positive colony visualized directly.

Isolation and sequencing by standard techniques identified a yeast EST fragment which served as the basis for additional database mining as described below.

#### 4. Assembly

The yeast EST fragment identified above was used to search various expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank, Merck/Wash U) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

A consensus DNA sequence was assembled relative to other EST sequences using phrap. The consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above as well as EST sequences proprietary to Genentech.

Based on this consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone encoding the particular PRO polypeptide. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, *Current Protocols in Molecular Biology*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

RNA for construction of the cDNA libraries was isolated from various human tissues. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel

electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD: pRK5B is a precursor of pRK5D that does not contain the SfiI site: Holmes *et al.*, *Science*, 253: 1278-1280 (1991)) in the unique XhoI and NotI sites.

*Signal algorithm:*

5 A proprietary signal sequence finding algorithm developed by Genentech, Inc was used upon Expressed Sequence Tags (ESTs) and on clustered and assembled EST fragments from public (e.g., GenBank) and/or private (Lifeseq<sup>®</sup>, Incyte Pharmaceuticals, Inc., Palo Alto, CA) databases. The signal sequence algorithm computes a secretion signal score based on the character of the DNA nucleotides surrounding the first and optionally the second methionine codon(s) (ATG) at the 5'-end of the sequence or sequence fragment under  
10 consideration. The nucleotides following the first ATG must code for at least 35 unambiguous amino acids without any stop codons. If the first ATG has the required amino acids, the second is not examined. If neither meets the requirement, the candidate sequence is not scored. In order to determine whether the EST sequence contains an authentic signal sequence, the DNA and corresponding amino acid sequences surrounding the ATG codon are scored using a set of seven sensors (evaluation parameters) known to be associated with secretion  
15 signals.

The above procedure resulted in the identification of EST sequences which were compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ<sup>®</sup>, Incyte Pharmaceuticals, Palo Alto, CA). The homology search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology*  
20 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). This resulted in the identification of additional EST sequences which either corresponded to full-length clones, which were examined and sequenced or served as a template for the creation of cloning oligonucleotides which were then used to screen various  
25 tissue libraries resulting in isolation of DNA encoding a native sequence PRO polypeptide.

A. Isolation of cDNA clones Encoding Human PRO184 (UNQ158)

The cDNA DNA28500 (Figure 1; SEQ ID NO:1) which encodes the PRO184 protein of Figure 2 (SEQ ID NO:2) is publicly available as GenBank accession number Q92914 and is also described in Smallwood, P.M. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 93: 9850-9857 (1996). The sequence is alternatively known as FGF-11 or  
30 FHF-3.

The entire nucleotide sequence of DNA28500 is shown in Figure 1 (SEQ ID NO:1). Clone DNA28500 (SEQ ID NO:1) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 731-733 and ending at the stop codon (TGA) at positions 1406-1408 (Fig. 1; SEQ ID NO:1), as indicated in bolded underline. The predicted PRO184 polypeptide precursor of Fig. 2 (SEQ ID NO:2) is 225  
35 amino acids in length, has a calculated molecular weight of 25005 daltons and a pI of 10.14.

Additional analysis of the PRO184 polypeptide of Figure 2 (SEQ ID NO:2) reveals the presence of a: a tyrosine kinase phosphorylation site at about amino acid residues 199-207; N-myristylation sites at about residues: 54-60, 89-95 and 131-137; HBGF/FGF family protein domains at about residues: 80-96, 104-155, 171-198 and a fibroblast growth factor domain at about residues 71-200.

40 B. Isolation of cDNA clones Encoding Human PRO212 (UNQ186)

Use of the ECD homology procedure described above from a human fetal lung library resulted in the identification of the full-length DNA sequence for DNA30942-1134 (Fig. 3; SEQ ID NO:3) and the derived PRO212 protein sequence of Fig. 4 (SEQ ID NO:4).

The PCR primers (forward and reverse) and probes used in the procedure were the following:

- 5 forward primer: 5'-CACGCTGGTTTCTGCTTGGAG-3' (SEQ ID NO:5)  
 reverse primer: 5'-AGCTGGTGCACAGGGTGTCTATG-3' (SEQ ID NO:6)  
 hybridization probe: (SEQ ID NO:7)  
 5'-CCCAGGCACCTTCTCAGCCAGCCAGCAGCTCCAGCTCAGAGCAGTGCCAGCCC-3'

- The entire nucleotide sequence of DNA30942-1134 is shown in Figure 3 (SEQ ID NO:3). Clone  
 10 DNA30942-1134 (SEQ ID NO:3) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 101-103 and ending at the stop codon (TGA) at positions 1001-1003 (Fig. 3; SEQ ID NO:3), as indicated in bolded underline. The predicted PRO212 polypeptide precursor of Fig. 4 (SEQ ID NO:4) is 300 amino acids long, has a calculated molecular weight of 32680 daltons and a pI of 8.70. It is believed that the PRO212 sequence of Fig. 4 (SEQ ID NO:4) lacks a transmembrane domain. It is also believed that amino  
 15 acids 1 to 215 of Fig. 4 (SEQ ID NO:4) represents an ECD which includes four cysteine rich domains (CRDs). A cDNA clone containing DNA30942-1134 (SEQ ID NO:3) has been deposited with ATCC on September 16, 1997 and has been assigned ATCC deposit no. 209254.

- Analysis of the PRO212 polypeptide of Figure 4 (SEQ ID NO:4) reveals the presence of a signal sequence at amino acid residues 1 to about 23, an N-glycosylation site at about residues 173-177, cAMP- and  
 20 cGMP-dependent protein kinase phosphorylation sites at about residues 63-67 and 259-263, tyrosine kinase phosphorylation site at about residues 28-37, N-myristoylation sites at about residues 156-162, 178-184, 207-213, 266-272 and 287-293, a TNFR/NGFR family cysteine-rich region protein domain at about residues 48-60, 149-161 and 168-175 and a death domain protein profile domain at residues 141-157.

#### C. Isolation of cDNA Clones Encoding Human PRO245 (UNO219)

- 25 Use of the ECD homology procedure described above in a human fetal liver library resulted in the isolation of the full-length DNA sequence for DNA35638-1141 (Figure 5, SEQ ID NO:8) and the derived PRO245 native sequence protein of Figure 6 (SEQ ID NO:9).

The PCR primers (forward and reverse) and hybridization probes synthesized for use with the above-described method were the following:

- 30 forward PCR primer 5'-ATCGTTGTGAAGTTAGTGCCCC-3' (SEQ ID NO:10)  
reverse PCR primer 5'-ACCTGCGATATCCAACAGAATTG-3' (SEQ ID NO:11)  
hybridization probe (SEQ ID NO:12)  
 5'-GGAAGAGGATACAGTCACTCTGGAAGTATTAGTGGCTCCAGCAGTTCC-3'

- The entire nucleotide sequence of DNA35638-1141 (SEQ ID NO:8) is shown in Figure 5. Clone  
 35 DNA35638 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 89-91 and ending at the stop codon (TAG) at nucleotide positions 1025-1027 (Fig. 5; SEQ ID NO:8). The predicted PRO245 polypeptide precursor of Figure 6 (SEQ ID NO:9) is 312 amino acids long, has a calculated molecular weight of 34,554 daltons and a pI of 9.39. A clone containing DNA35638-1141 (SEQ ID NO:8) has been deposited with ATCC on September 16, 1997 and is assigned ATCC deposit no. 209265.

Further analysis of the PRO245 polypeptide of Figure 6 (SEQ ID NO:9) reveals the presence of: a signal peptide at amino acid residues 1 to about 20, a transmembrane domain at about amino acid residues 237-258, an N-glycosylation site at about amino acid residues 98-102, 187-191, 236-240 and 277-281, N-myristoylation sites at about amino acids residues 82-188, 239-245, 255-261, 257-263, 305-311, an amidation site at about amino acid residues 226-230 and an immunoglobulin domain at about amino acid residues 148-216.

D. Isolation of cDNA clones Encoding Human PRO266 (UNQ233)

Use of the ECD homology procedure described above in a human fetal brain library resulted in the isolation of the full-length DNA sequence for DNA37150-1178 (Figure 7, SEQ ID NO:13) and the derived PRO266 native sequence protein of Figure 8 (SEQ ID NO:14).

The PCR primer (forward and reverse) and hybridization probe synthesized were the following:

forward PCR primer: 5'-GTTGGATCTGGGCAACAATAAC-3' (SEQ ID NO: 15)

reverse PCR primer: 5'-ATTGTTGTGCAGGCTGAGTTTAAG-3' (SEQ ID NO: 16)

hybridization probe:

5'-GGTGGCTATACATGGATAGCAATTACCTGGACACGCTGTCCCGGG-3' (SEQ ID NO: 17)

Clone DNA37150-1178 (SEQ ID NO:13) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 167-169 and ending at the stop codon (TAA) at nucleotide positions 2255-2257 (Figure 7), as indicated by bolded underline. The predicted PRO266 precursor of Figure 8 (SEQ ID NO:14) is 696 amino acids long, has a calculated molecular weight of 77735 daltons and a pI of 6.36. A cDNA clone including DNA37150-1178 (SEQ ID NO:13) has been deposited with the ATCC on October 17, 1997 and has been assigned ATCC deposit no. 209401.

Analysis of the PRO266 polypeptide of Figure 8 (SEQ ID NO:14) reveals: a signal peptide at about amino acid residues 1 to about 15, a transmembrane at about residues 616-639, an N-glycosylation site at about residues: 18-22, 253-257, 363-367, 416-420, 595-599, 655-659, a cAMP- and cGMP-dependent protein kinase phosphorylation site at about residues 122-126 and 646-650, N-myristoylation sites at about residues 17-23, 67-73, 100-106, 302-308, 328-334, 343-349, 354-360, 465-471, 493-499, 598-604, 603-609, a prokaryotic membrane lipoprotein lipid attachment site at about residues 337-348, an arthropod defensin protein domain at about residues 216-222, a leucine rich repeat at about residues 179-199 and a leucine rich repeat C-terminal domain at about residues 212-262 and 529-579.

E. Isolation of cDNA clones Encoding Human PRO306 (UNQ269)

Use of the ECD homology procedure described above in a human fetal kidney library resulted in the isolation of the full-length DNA sequence for DNA39984-1221 (Fig. 9; SEQ ID NO:18) and the derived PRO306 native sequence protein of Figure 10 (SEQ ID NO:19).

The PCR primers (forward and reverse) and hybridization probe synthesized were the following:

forward PCR primer (.f1): 5'-CAGGTCTGAACCCAGACCACGATGC-3' (SEQ ID NO:20)

forward PCR primer (.f2): 5'-GCCACATGGCCCAGCTTG-3' (SEQ ID NO:21)

forward PCR primer (.f3): 5'-GAGACGGAGGAAGCAGGC-3' (SEQ ID NO:22)

forward PCR primer (.f1a): 5'-GGCCACACTTACAGCTCTG-3' (SEQ ID NO:23)

reverse PCR primer (.r1): 5'-AGCCGGCTTCTGAGGGCGTCTACC-3' (SEQ ID NO:24)

hybridization probe:

5'-TGGTGCTGCCGCTGCTGCTCTGGCCGCGGCAGCCCTGGCCGAAG-3' (SEQ ID NO:25)



Clone DNA39984-1221 (SEQ ID NO:18) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 199-201 and ending at the stop codon (TAG) at nucleotide positions 1471-1473 (Figure 9), as indicated by bolded underline. The predicted PRO306 polypeptide precursor of Figure 10 (SEQ ID NO:19) is 424 amino acids long, has a calculated molecular weight of 46,332 daltons and a pI of 4.76 (Figure 10). A cDNA clone including DNA39984-1221 (SEQ ID NO:18) has been deposited with the ATCC on November 7, 1997 and is assigned ATCC deposit number 209435.

Analysis of the PRO306 polypeptide of Figure 10 (SEQ ID NO:19) reveals the presence of a signal sequence at residues 1 to about 22, an N-glycosylation site about residues 225-229, a glycosaminoglycan site at about residues 388-392, a tyrosine kinase phosphorylation site about residues 62-70, N-myristoylation sites at about residues 28-34, 130-136, 201-207, 226-232, 237-243, 362-368, 372-378 and 387-393, a thyroglobulin type-1 repeat domain about residues 335-348, a kazal serine protease domain at about residues 140-162, an osteonectin domain protein signature at about residues 283 to 317 and a CTF-NF-1 protein domain at residues 324-358.

#### F. Isolation of cDNA clones Encoding Human PRO333 (UNO294)

Use of the ECD homology procedure in combination with an *in vivo* cloning procedure resulted in the identification of the partial length sequence DNA41374-1312 (SEQ ID NO:26, Figure 11).

Clone DNA41374-1312 (SEQ ID NO:26) contains an incomplete open reading frame with an apparent translation termination site (*i.e.*, stop codon, TGA) at nucleotide residues 1185-1187, as indicated in bolded underline. The predicted partial length PRO333 polypeptide of Figure 12 (SEQ ID NO:27) is 394 amino acids long, a calculate molecular weight of 43,725 daltons and a pI of 8.36.

Analysis of the PRO333 (SEQ ID NO:27) polypeptide of Figure 12 reveals a signal sequence at about amino acid residues 1-14, a transmembrane domain at about residues 359-376, N-myristoylation sites at about amino acid residues 166-172, 206-212, 217-223, 246-252, 308-314, 312-318, 361-367 and an immunoglobulin and major histocompatibility complex proteins signature at amino acid residues 315-323. A cDNA clone containing DNA41374-1312 has been deposited with the ATCC on \_\_\_\_\_ and as assigned ATCC deposit number \_\_\_\_\_.

#### G. Isolation of cDNA clones Encoding Human PRO526 (UNO330)

Use of the ECD homology procedure described above in a human fetal liver library resulted in the identification of the full-length DNA sequence DNA44184-1319 (Fig. 13, SEQ ID NO:28) and the derived PRO526 native sequence protein of Figure 14 (SEQ ID NO:29).

The PCR primers (forward and reverse) and hybridization probes synthesized were the following:

forward PCR primer: 5'-TGGCTGCCCTGCAGTACCTCTACC-3' (SEQ ID NO:30)

reverse PCR primer: 5'-CCCTGCAGGTCATTGGCAGCTAGG-3' (SEQ ID NO:31)

hybridization probe: (SEQ ID NO:32)

5'-AGGCACTGCCTGATGACACCTTCCGCGACCTGGGCAACCTCACAC-3'

Clone DNA44184-1319 (SEQ ID NO:28) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 514-516 and ending at the stop codon (TGA) at nucleotide positions 1933-1935 (Figure 13), as indicated by bolded underline. The predicted PRO526 polypeptide precursor of Figure 14 (SEQ ID NO:29) is 473 amino acids long. The PRO526 (SEQ ID NO:29) protein shown in Figure 14 has an estimated molecular weight of about 50708 daltons and a pI of about 9.28. A cDNA clone

containing DNA44184-1319 has been deposited with the ATCC on 26 March 1998 and is assigned deposit number 209704.

Analysis of the PRO526 polypeptide of Figure 14 (SEQ ID NO:29) reveals that the signal peptide sequence is at about amino acids 1-26. A leucine zipper pattern is at about amino acids 135-156. A glycosaminoglycan attachment is at about amino acids 436-439. N-glycosylation sites are at about amino acids 82-85, 179-182, 237-240 and 423-426. A von Willebrand factor (VWF) type C domain(s) is found at about amino acids 411-425. The skilled artisan can understand which nucleotides correspond to these amino acids based on the sequences provided herein.

#### H. Isolation of cDNA clones Encoding Human PRO381 (UNQ322)

Use of the ECD homology procedure described above in a human fetal kidney library resulted in the identification of the full length DNA sequence DNA44194-1317 (Fig. 15, SEQ ID NO:33) and the derived PRO381 native sequence protein of Figure 16 (SEQ ID NO:34).

The forward and reverse PCR primers and the hybridization probe used were the following:

Forward PCR primer (39651.f1): (SEQ ID NO:35)

5'-CTTTCCTTGCTTCAGCAACATGAGGC-3'

Reverse PCR primer (39651.r1): (SEQ ID NO:36)

5'-GCCCAGAGCAGGAGGAATGATGAGC-3'

hybridization probe (39651.p1): (SEQ ID NO:37)

5'-GTGGAACGCGGTCTTGACTCTGTCGTCACCTTCTTTGATTGGGGCTTTG-3'

Clone DNA44194-1317 (SEQ ID NO:33) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 174-176 and ending at the stop codon (TAG) at nucleotide positions 807-809 (Fig. 15), as indicated by bolded underline. The predicted PRO381 polypeptide precursor of Figure 16 (SEQ ID NO:34) is 211 amino acids long, has a calculated molecular weight of 24,172 daltons and has a pI of 5.99. The PRO381 (SEQ ID NO:34) protein shown in Figure 16 has the following features: a signal peptide from about amino acid residues 1 to about 20, a potential N-glycosylation site at about amino acid residue 156, potential casein kinase phosphorylation sites from about amino acid residues 143 to about 146, about residues 156 to about 159, about residues 178 to about 181, about residues 200 to about 203, an endoplasmic reticulum targeting sequence from about amino acid residues 78 to about 114 and from about residues 118 to about 131, EF-hand calcium binding domain from about amino acid residues 140 to about 159, and an S-100/ICaBP type calcium binding domain from about amino acid residues 183 to about 203. A cDNA clone containing DNA44194-1317 (SEQ ID NO:33) has been deposited with the ATCC on April 28, 1998 and is assigned deposit number 209808.

#### I. Isolation of cDNA clones Encoding Human PRO364 (UNQ319)

Use of the ECD homology procedure described above in a human small intestine library resulted in the identification of an expressed sequence tag (EST) (Incyte EST No. 3003460) that encoded a polypeptide which showed homology to members of the tumor necrosis factor receptor (TNFR) family of polypeptides.

A consensus DNA sequence was then assembled relative to the Incyte 3003460 EST in a manner similar to that used in the ECD homology procedure which resulted in the isolation of the full-length DNA sequence DNA47365-1206 (Fig. 17, SEQ ID NO:38) and the derived PRO364 native sequence protein of Figure 18 (SEQ ID NO:39).

The PCR primers (forward and reverse) and hybridization probes synthesized for use in the above-described screening procedure were:

forward PCR primer (44825.f1): 5'-CACAGCACGGGGCGATGGG-3' (SEQ ID NO:40)

forward PCR primer (44825.f2): 5'-GCTCTGCGTTCTGCTCTG-3' (SEQ ID NO:41)

5 forward PCR primer (44825.G1TR.f):

5'-GGCACAGCACGGGGCGATGGGCGCGTTT-3' (SEQ ID NO:42)

reverse PCR primer (44825.r1): 5'-CTGGTCACTGCCACCTTCCTGCAC-3' (SEQ ID NO:43)

reverse PCR primer (44825.r2): 5'-CGCTGACCCAGGCTGAG-3' (SEQ ID NO:44)

reverse PCR primer (44825.G1TR.r):

10 5'-GAAGGTCCCCGAGGCACAGTCGATACA-3' (SEQ ID NO:45)

hybridization probe (44825.pl):

5'-GAGGAGTGCTGTCCGAGTGGGACTGCATGTGTGTCCAGC-3' (SEQ ID NO:46)

hybridization probe (44825.G1TR.p):

5'-AGCCTGGGTACGCGCCCCACGGGGTCCCGGGTGCGGCC-3' (SEQ ID NO:47)

15 Clone DNA47365-1206 (SEQ ID NO:38) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 121-123 and ending at the stop codon (TGA) at nucleotide positions 844-846 (Figure 17), as indicated by bolded underline. The predicted PRO364 polypeptide precursor of Figure 18 (SEQ ID NO:39) is 241 amino acids long. The PRO364 (SEQ ID NO:39) protein shown in Figure 18 has an estimated molecular weight of about 26,000 daltons and a pI of about 6.34. A potential N-glycosylation sites exists between amino acids 146 and 149 of the amino acid sequence shown in Figure 18. A putative signal sequence is from amino acids 1 to 25 and a potential transmembrane domain exists between amino acids 162 to 180 of the sequence shown in Figure 18. A cDNA clone containing DNA47365-1206 has been deposited with ATCC on November 7, 1997 and is assigned ATCC Deposit No. ATCC 209436.

#### J. Isolation of cDNA clones Encoding Human PRO356 (UNQ313)(NL4)

25 An expressed sequence tag (EST) DNA database (LIFESEQ<sup>®</sup>, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST (#2939340) was identified which showed homology to human TIE-2 L1 and TIE-2 L2.

Based on the EST, a pair of PCR primers (forward and reverse), and a probe were synthesized:

NL4,5-1: 5'-TTCAGCACCAAGGACAAGGACAATGACAACT-3' (SEQ ID NO:50)

30 NL4,3-1: 5'-TGTGCACACTTGTCGAAGCAGTTGTCATTGTC-3' (SEQ ID NO:51)

NL4,3-3: 5'-GTAGTACACTCCATTGAGGTTGG-3' (SEQ ID NO:52).

Oligo dT primed cDNA libraries were prepared from uterus mRNA purchased from Clontech, Inc. (Palo Alto, CA, USA, catalog # 6537-1) in the vector pRK5D using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System). pRK5D is a cloning vector that has an sp6 transcription initiation site followed by an SfiI restriction enzyme site preceding the XhoI/NotI cDNA cloning sites. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized to greater than 1000 bp appropriately by gel electrophoresis, and cloned in a defined orientation into XhoI/NotI-cleaved pRK5D.

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO356 gene using the probe oligonucleotide and one of the PCR primers.

DNA sequencing of the clones isolated as described above gave a full-length DNA sequence  
5 DNA47470-1130 (SEQ ID NO:48) and the derived PRO356 protein (SEQ ID NO:49) shown in Figure 20.

The entire nucleotide sequence of DNA47470-1130 is shown in Figure 19 (SEQ ID NO:48). Clone DNA47470-1130 (SEQ ID NO:48) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 215-217, and a TAA stop codon at nucleotide positions 1038-1040, as indicated by bolded underline. The predicted PRO356 polypeptide shown in Figure 20 is 346 amino acids long (SEQ ID  
10 NO:49), has a calculated molecular weight of 40,018 daltons and a pI of 8.19. A cDNA clone containing DNA47470-1130 (SEQ ID NO:48) has been deposited with ATCC on October 28, 1997 and is assigned ATCC deposit no. 209422.

Further analysis of the PRO356 polypeptide of Figure 20 (SEQ ID NO:49) reveals: a signal peptide at amino acid residues 1 to about 26, N-glycosylation sites at about residues 58-62, 253-257 and 267-271,  
15 glycosaminoglycan attachment sites at residues 167-171, a cAMP- and cGMP-dependent protein kinase phosphorylation site at about residues 176-180, N-myristoylation sites at about residues 168-174, 196-202, 241-247, 252-258, 256-262, 327-333, a cell attachment sequence at about residues 199-202, and fibrinogen beta and gamma chains C-terminal domain proteins at about residues 160-198, 201-210, 219-256, 266-279, 283-313.

K. Isolation of cDNA clones Encoding Human PRO719 (UNQ387)

20 Use of the ECD homology procedure identified above in a human placenta tissue library resulted in the isolation of the full-length DNA sequence DNA49646-1327 (Fig. 21, SEQ ID NO:53) and the derived PRO719 native sequence protein of Figure 22 (SEQ ID NO:54).

The PCR primers (forward and reverse) and hybridization probe synthesized were:

forward PCR primer (44851.f1): 5'-GTGAGCATGAGCGAGCCGTCCAC-3' (SEQ ID NO:55)  
25 reverse PCR primer (44851.r1): 5'-GCTATTACAACGGTTCTTGCGGCAGC-3' (SEQ ID NO:56)  
hybridization probe (44851.p1): (SEQ ID NO:57)  
5'-TTGACTCTCTGGTGAATCAGGACAAGCCGAGTTTGCCTTCCAG-3'

Clone DNA49646-1327 (Fig. 21, SEQ ID NO:53) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 223-225 and ending at the stop codon (TGA) at  
30 nucleotide positions 1285-1287 (Figure 21), as indicated by bolded underline. The predicted PRO719 polypeptide precursor of Figure 22 (SEQ ID NO:54) is 354 amino acids long, has an estimated molecular weight of about 39,362 daltons and a pI of about 8.35. A clone containing DNA49646-1327 (SEQ ID NO:53) has been deposited with the ATCC on March 26, 1998 (under the designation DNA49646-1327) and has been assigned ATCC deposit number 209705.

35 Analysis of the PRO719 protein sequence of Figure 22 (SEQ ID NO:54) reveals a signal peptide at amino acid residues 1 to about 16, a lipase/serine active site at about residues 163-173, N-glycosylation sites at about residues 80-84 and 136-140, a cAMP- and cGMP-dependent protein kinase phosphorylation site at about residues 206-210 and 329-333 and N-myristoylation sites at about residues 63-69, 96-102, 171-177, 191-197, 227-233, 251-157, 306-312 and 346-352.

40 L. Isolation of cDNA clones Encoding Human PRO861 (UNQ423)

The cDNA DNA50798 (Figure 23; SEQ ID NO:58) which encodes the PRO861 protein of Figure 24 (SEQ ID NO:59) is publicly available as GenBank accession number P22692 and is also described in Latour, D. *et al.*, *Mol. Endocrinol.* 4: 1806-1814 (1990); Shimasaki, S. *et al.*, *Mol. Endocrinol.* 4: 1451-1458 (1990); Kiefer, M.C. *et al.*, *J. Biol. Chem.* 266: 9043-9049 (1991); Culouscou *et al.*, *Cancer Res.* 51: 2813-2819 (1991).

5 The sequence is alternatively known as insulin-like growth factor binding protein 4 precursor.

The entire nucleotide sequence of DNA50798 is shown in Figure 23 (SEQ ID NO:58). Clone DNA50798 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 265-267 and ending at the stop codon (TGA) at positions 1039-1041 (Fig. 23; SEQ ID NO:58), as indicated in bolded underline. The predicted PRO861 polypeptide precursor of Fig. 24 (SEQ ID NO:59) is 258  
10 amino acids in length, has a calculated molecular weight of 27934 daltons and a pI of 7.23.

Additional analysis of the PRO861 polypeptide of Figure 24 reveals the presence of: a signal sequence at amino acid residues 1 to about 21, an N-glycosylation site at about residues 125-129, a tyrosine kinase phosphorylation site at about residues 191-198, N-myristoylation sites at about residues 52-58, 54-60, 64-70, 96-102 and 172-178, insulin-like growth factor binding protein domains at about residues 46-80, 201-229 and  
15 thyroglobulin type-1 repeat domains at residues 52-100, 202-215 and 220-231.

#### M. Isolation of cDNA clones Encoding Mouse PRO769 (UNQ407)

A public expressed sequence tag (EST) DNA databases (Merck/Washington University) was searched with the full-length murine m-FIZZ1 DNA (DNA 53517) cDNA and the EST W42069 was identified.

The full-length clones corresponding to the EST fragment W42069 was obtained from Incyte  
20 Pharmaceuticals (Palo Alto, California), and sequenced in the entirety, which ultimately resulted in the identification of the full length nucleotide sequence DNA54231-1366-1 (SEQ ID NO:60).

The nucleotide sequence corresponding to the full length, native sequence PRO769 clone is shown in Figure 25. This clone, designated DNA 54231-1366-1 (SEQ ID NO:60) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 75-77 and ending at the stop codon (TGA) at  
25 residues 417-419, as indicated by bolded underline (Fig. 25). The predicted PRO769 polypeptide precursor (including a signal sequence of 10 amino acids)(SEQ ID NO:61) is 114 amino acids long, has a calculated molecular weight of 12,492 daltons and a pI of 8.19. Based on its homology to m-FIZZ1 (34%, using the ALIGN software) the protein was designated m-FIZZ3. A clone containing DNA54231-1366-1 (designated DNA54231-1366-1) has been deposited with ATCC on April 23, 1998 and has been assigned ATCC deposit no.  
30 209804.

Additional analysis of the PRO769 polypeptide of Figure 26 (SEQ ID NO:61) reveals the presence of a signal peptide at amino acid residues 1 to about 20, a leucine zipper pattern at about residues 4-25, an N-glycosylation site at about residues 3-6 and a DNA polymerase family B protein domain at residues 39-48.

#### Identification and cloning of m-FIZZ1 (DNA53517)

35 *Mouse asthma model* Female Balb/C mice, 6 to 8 weeks of age, were separated into two experimental groups: controls and asthmatics. The asthmatic group was immunized intraperitoneally with 10 µg ovalbumin + 1 mg alum, while the control group was not. Two weeks later, mice were exposed daily to an aerosol of 10 mg/ml ovalbumin in PBS aerosolized with a UltraNeb nebulizer (DeVilbiss) at the rate of 2 ml/min for 30 min each day, for 7 consecutive days. One day after the last aerosol challenge, whole blood,

serum and bronchoalveolar lavage (BAL) samples were collected and the lungs were harvested and preserved for histological examination, immuno-histochemistry and *in situ* hybridization.

*Gel electrophoresis of BAL samples* Examination of the BAL samples by gel electrophoresis on a 16% Tricine gel shows that a low molecular weight protein is expressed in the BAL samples from asthmatic mice but not in the BAL samples from control mice. This low molecular weight protein was termed m-FIZZ1 and was seen to co-migrate with a 8300 Dalton marker protein.

*Partial protein sequence* The protein of interest was transferred upon a PVDF membrane and sequenced by Edman degradation. This sequence served as a template for the preparation of various cloning oligos as described below.

*Partial cDNA sequence* We designed two degenerate oligonucleotide PCR primers corresponding to the putative DNA sequence for the first 7 and the last 7 amino acids of the partial protein sequence.

Oligo #1:

5'-ACA AAC GCG TGA YGA RAC NAT HGA RAT-3' (SEQ ID NO:62)

Oligo # 2:

5'-TGG TGC ATG CGG RTA RTT NGC NGG RTT-3' (SEQ ID NO:63)

cDNA prepared from the lungs of normal mice was used as a template for the PCR reaction which yielded an 88 bp product. This 88 bp product contained 54 known base pairs, encoding the PCR primers, and 34 novel base pairs, and encoded another partial mFIZZ-1 sequence.

*Full length cDNA clone* This second partial sequence was used to design primers which were ultimately successful in obtaining the full length FIZZ clone (DNA53517) by RT-PCR of mouse lung poly(A)<sup>+</sup> RNA.

Oligo #3:

5'-ACA AAC GCG TGC TGG AGA ATA AGG TCA AGG-3' (SEQ ID NO:64)

This oligo was used as an RT-PCR primer in combination with 5' and 3' amplimers from Clontech.

Oligo #4:

5'-ACT AAC GCG TAG GCT AAG GAA CTT CTT GCC-3' (SEQ ID NO: 65)

This oligo was used as an RT-PCR primer in combination with oligo d(T).

#### N. Isolation of cDNA clones Encoding Human PRO788 (UNQ430)

Use of the ECD homology procedure identified above resulted in the identification of the partial length EST sequence 2777282. Further analysis of the corresponding full-length sequence resulted in the identification of DNA56405-1357 (SEQ ID NO:66) and the derived native sequence PRO788 protein (SEQ ID NO:67) of Figure 28.

Clone DNA56405-1357 (SEQ ID NO:66) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 84-86 and ending at the stop codon (TAG) at nucleotide positions 459-461 (Figure 27), as indicated by bolded underline. The predicted native sequence PRO788 polypeptide precursor (SEQ ID NO:67) is 125 amino acids long (Figure 28), has a calculated molecular weight of 13,115 daltons and a pI of 5.90. The PRO788 (SEQ ID NO:67) protein shown in Figure 28 has an estimated molecular weight of about 13115 and a pI of about 5.90. A clone containing DNA56405-1357 (SEQ ID NO:66) has been deposited with the ATCC on May 6, 1998 and has been assigned deposit number 209849. In the event of a discrepancy in the nucleotide sequence of the deposit and the sequences disclosed herein, it is understood

that the deposited clone contains the correct sequence. It is further understood that the methodology of sequencing for the sequences provided herein are based on known sequencing techniques.

Analysis of the PRO788 polypeptide (SEQ ID NO:67) shown in Figure 28 reveals a signal peptide at about amino acids 1-17 and an N-glycosylation site is at about amino acid 46.

5 O. Isolation of cDNA clones Encoding Human PRO826 (UNQ467)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence 47283. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Merck EST sequence W69233. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA57694-1341 (Fig. 29; SEQ ID NO:68) and the derived PRO826 native sequence protein of Figure 30 (SEQ ID NO:69).

Clone DNA57694-1341 (SEQ ID NO:68) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 13-15 and ending at the stop codon (TGA) at nucleotide positions 310-312 (Figure 29), as indicated by bolded underline. The predicted PRO826 polypeptide precursor of Figure 30 (SEQ ID NO:69) is 99 amino acids long. The PRO826 (SEQ ID NO:69) protein shown in Figure 30 has an estimated molecular weight of about 11050 daltons and a pI of about 7.47.

Analysis of the full-length PRO826 sequence shown in Figure 30 (SEQ ID NO:69) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 22, potential N-myristoylation sites from about amino acid 22 to about amino acid 27 and from about amino acid 90 to about amino acid 95 and an amino acid sequence block having homology to peroxidase from about amino acid 16 to about amino acid 48.

A cDNA clone containing DNA57694-1341 has been deposited with ATCC on June 23, 1998 and is assigned ATCC deposit no. 203017.

P. Isolation of cDNA clones Encoding Human PRO982 (UNQ483)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence no. 43715. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Merck EST No. AA024389. Further examination and sequencing of the full-length clone corresponding to this EST resulted in the identification of the full-length sequence DNA57700-1408 (Fig. 31, SEQ ID NO:70) and the derived PRO982 native sequence protein of Figure 32 (SEQ ID NO:71).

The DNA57700-1408 sequence of Figure 31 (SEQ ID NO:70) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 26-28 and ending at the stop codon (TAA) at nucleotide positions 401-403, as indicated by bolded underline. The predicted PRO982 polypeptide precursor of Figure 32 (SEQ ID NO:71) is 125 amino acids in length, has a calculated molecular weight of approximately 14,198 daltons and an estimated pI of approximately 9.01. Further analysis of the PRO982 (SEQ ID NO:71) polypeptide of Figure 32 reveals a signal peptide from about amino acid residues 1 to about 21, N-myristoylation sites at about residues 33-39 and 70-76 and a potential anaphylatoxin domain from about amino acid residue 1 to about residue 59. A cDNA clone containing DNA57700-1408 (SEQ ID NO:70) was deposited with the ATCC on January 12, 1999 under the designation DNA57700-1408 and is assigned ATCC deposit No. 203583.

Q. Isolation of cDNA clones Encoding Human PRO779 (UNQ455)(Apo-3)

Human fetal heart and human fetal lung lgt10 bacteriophage cDNA libraries (both purchased from Clontech) were screened by hybridization with synthetic oligonucleotide probes based on an EST (Genbank locus W71984), which showed some degree of homology to the intracellular domain (ICD) of human TNFR1 and CD95. W71984 is a 523 bp EST, which in its -1 reading frame has 27 identities to a 43 amino acid long sequence in the ICD of human TNFR1. The oligonucleotide probes used in the screening were 27 and 25 bp long, respectively, with the following sequences:

5'-GGCGCTCTGGTGGCCCTTGCAGAAGCC-3' (SEQ ID NO:74)  
 10 5'-TTCGGCCGAGAAGTTGAGAAATGTC-3' (SEQ ID NO:75)

Hybridization was done with a 1:1 mixture of the two probes overnight at room temperature in buffer containing 20% formamide, 5X SSC, 10% dextran sulfate, 0.1% NaPiPO<sub>4</sub>, 0.05 M NaPO<sub>4</sub>, 0.05 mg salmon sperm DNA, and 0.1% sodium dodecyl sulfate (SDS), followed consecutively by one wash at room temperature in 6X SSC, two washes at 37°C in 1X SSC/0.1% SDS, two washes at 37°C in 0.5X SSC/0.1% SDS, and two washes at 37°C in 0.2X SSC/0.1% SDS. One positive clone from each of the fetal heart (FH20A.57) and fetal lung (FL8A.53) libraries were confirmed to be specific by PCR using the respective above hybridization probes as primers. Single phage plaques containing each of the positive clones were isolated by limiting dilution and the DNA was purified using a Wizard lambda prep DNA purification kit (Promega).

The cDNA inserts were excised from the lambda vector arms by digestion with EcoRI, gel-purified, and subcloned into pRK5 that was predigested with EcoRI. The clones were then sequenced in entirety resulting in the isolation and identification of the full-length clone DNA58801-1052 (Fig. 33; SEQ ID NO:72), alternatively referred to as clone FH20.57, and the derived PRO770 native sequence protein of Figure 34 (SEQ ID NO:73).

Clone FH20A.57 (also referred to as Apo 3 clone FH20.57 deposited as ATCC 55820, as indicated below) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 103-105 and ending at the stop codon (TGA) at nucleotide positions 1354-1356 (Figure 33), as indicated by bolded underline. The predicted PRO779 polypeptide precursor of Figure 34 (SEQ ID NO:73) is 417 amino acids long and has a calculated molecular weight of about 45385 daltons and a pI of about 6.4.

A cDNA clone containing DNA58801-1052 (SEQ ID NO:72) has been deposited with the ATCC under the designation FH20.57 on Sept. 5, 1996 and has been assigned ATCC deposit No. 55820. In the event of a discrepancy between the sequence disclosed herein and the sequence of the deposit, it is understood that the deposited clone contains the correct sequence, and that the sequences provided herein are provided using known sequencing techniques.

Additional analysis of the PRO779 polypeptide of Figure 34 (SEQ ID NO:73) reveals: a signal peptide at amino acid residues 1 to about 24, a transmembrane domain at about residues 199-218, N-glycosylation site at about residues 67-71 and 106-110, cAMP- and cGMP-dependent protein kinase phosphorylation sites at about residues 157-161, a tyrosine kinase phosphorylation site at about residues 370-377, N-myristoylation sites at about residues 44-50, 50-56, 66-72, 116-122, 217-223, 355-361, 391-397, 401-407, prokaryotic membrane lipoprotein lipid attachment site at about residues 177-188, a death domain at about residues 333-413 and a TNFR/NGFR family cysteine-rich region protein domain at residues 47-59.



R. Isolation of cDNA clones Encoding Human PRO1068 (UNQ525)

Use of the signal algorithm procedure described above resulted in the identification of the Incyte cluster sequence no. 141736. This sequence was then compared to a variety of various EST databases described under signal algorithm procedure above, and further resulted in the identification of Incyte EST clone no. 004974. The full-length clone corresponding to this EST resulted in the identification of the full-length sequence DNA59214-1449 (Fig. 35, SEQ ID NO:76) and the derived PRO1068 native sequence protein of Figure 36 (SEQ ID NO:77).

The DNA59214-1449 sequence of Figure 35 (SEQ ID NO:76) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 42-44 and ending at the stop codon (TGA) at nucleotide positions 414-416, as indicated by bolded underline. The predicted PRO1068 polypeptide precursor of Figure 36 (SEQ ID NO:77) is 124 amino acids long, has a calculated molecular weight of about 14,284 Daltons and an estimated pI of approximately 8.14.

Further analysis of the PRO1068 (SEQ ID NO:77) polypeptide of Figure 36 reveals a signal peptide from about amino acid residues 1 to about 20, a urotension II signature sequence at about amino acids 118-124, a cell attachment sequence at about amino acids 64-67, and a potential cAMP- and cGMP-dependent protein kinase phosphorylation site at about amino acids 112-116 and N-myristoylation sites at about residues 61-67 and 92-98. A cDNA clone containing DNA59214-1449 (SEQ ID NO:76) was deposited with the ATCC on July 1, 1998 under the designation DNA59214-1449 and is assigned ATCC deposit No. 203046.

S. Isolation of cDNA clones Encoding Human PRO1031 (UNQ516)

Use of the ECD homology procedure described above resulted in the identification of the EST sequence Merck W74558 (clone 344649). The corresponding full-length clone was examined and sequenced resulting in the isolation of DNA sequencing gave the full-length DNA sequence DNA59294-1381 (Fig. 37, SEQ ID NO:78) and the derived PRO1031 native sequence protein of Figure 38 (SEQ ID NO:79).

Clone DNA59294-1381 (SEQ ID NO:78) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 42-44 and ending at the stop codon (TGA) at nucleotide positions 582-584 (Figure 37), as indicated by bolded underline. The predicted PRO1031 polypeptide precursor of Figure 38 (SEQ ID NO:79) is 180 amino acids long. The PRO1031 protein (SEQ ID NO:79) shown in Figure 38 has an estimated molecular weight of about 20437 and a pI of about 9.58. A cDNA clone containing DNA59294-1381 (SEQ ID NO:78) has been deposited with the ATCC on May 14, 1998 under the designation DNA59294-1381 and has been assigned deposit number 209866. In the event of a discrepancy between the sequence as disclosed herein and the sequence of the deposit, it is understood that the deposited clone contains the correct sequence, and the sequences provided herein were produced using known sequencing techniques.

Analysis of the amino acid sequence of the PRO1031 protein of Figure 38 (SEQ ID NO:79) reveals the putative signal peptide at about amino acid residues 1-20, an N-glycosylation site is at about amino acid residue 75-79. A region having sequence identity with IL-17 is at about amino acid residues 96-180.

T. Isolation of cDNA clones Encoding Human PRO1157 (UNQ587)

Use of the signal algorithm procedure described above resulted in the identification of an Incyte EST clone sequence from the LIFESEQ® database, designated 65816. This sequence was then compared to a variety of various EST databases described under the signal algorithm procedure above, and further resulted in the identification of Merck EST No. AA516481. Further examination and sequencing of the full-length clone

corresponding to this EST (No. 955952) resulted in the identification of the full-length sequence DNA60292-1506 (Fig. 39, SEQ ID NO:80) and the derived PRO1157 native sequence protein of Figure 40 (SEQ ID NO:81).

The cDNA DNA60292-1506 (SEQ ID NO:80) sequence shown in Figure 39 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 56-58 and ending at the stop codon (TGA) at nucleotide positions 332-334, as indicated by bolded underline. The predicted PRO1157 polypeptide precursor shown in Figure 40 (SEQ ID NO:81) is 92 amino acids long, has a calculated molecular weight of approximately 9,360 Daltons and an estimated pI of approximately 9.17.

Further analysis of the PRO1157 (SEQ ID NO:81) sequence reveals: a signal peptide at amino acid residues 1 to about 18, a transmembrane domain at about residues 51-70, a glycosaminoglycan attachment site at about residues 40-44, N-myristoylation sites at about residues 34-40, 37-43, 52-58 and a prokaryotic membrane lipoprotein lipid attachment site at about residues 29-40. A cDNA clone containing DNA60292-1506 (SEQ ID NO:80) was deposited with the ATCC on December 15, 1998 under the designation DNA60292-1506 and has been assigned deposit number 203540.

#### 15 U. Isolation of cDNA clones Encoding Human PRO1159 (UNQ589)

Use of the signal algorithm procedure described above resulted in the identification of EST cluster sequence 77245, which was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST no. 376776. Analysis of the full-length clone corresponding to this EST resulted in the identification of the full-length sequence DNA60627-1508 (Fig. 41, SEQ ID NO:82) and the derived PRO1159 native sequence protein of Figure 42 (SEQ ID NO:83).

Clone DNA60627-1508 (SEQ ID NO:82) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 92-94 and ending at the stop codon (TAG) at nucleotide positions 362-364 (Figure 41), as indicated by bolded underline. The predicted PRO1159 polypeptide precursor of Figure 42 (SEQ ID NO:83) is 90 amino acids long. The PRO1159 (SEQ ID NO:83) protein shown in Figure 42 has an estimated molecular weight of about 9,840 daltons and a pI of about 10.13.

Analysis of the PRO1159 (SEQ ID NO:83) sequence shown in Figure 42 evidences the presence of the following: a signal peptide from about amino acid residue 1 to about residue 15 and a potential N-glycosylation site at about amino acid residue 38. Clone DNA60627-1508 (SEQ ID NO:82) has been deposited with ATCC on August 4, 1998 and is assigned ATCC deposit no. 203092.

#### 30 V. Isolation of cDNA clones Encoding Human PRO1475 (UNQ746)

Use of the ECD homology procedure described above in a human fetal brain tissue library resulted in the isolation of the full-length DNA sequence DNA61185-1646 (Fig. 43, SEQ ID NO:84) and the derived PRO1475 native sequence protein of Figure 44 (SEQ ID NO:85).

The PCR primers (forward and reverse) and hybridization probe synthesized were:

<u>forward PCR primer</u> (45639.f1):	5'-GATGGCAAACGTTGTGTTTGACACG-3'	(SEQ ID NO:86)
<u>forward PCR primer</u> (45639.f2):	5'-CCTCAACCAGGCCACGGGCCAC-3'	(SEQ ID NO:87)
<u>reverse PCR primer</u> (45639.r1):	5'-CCCAGGCAGAGATGCAGTACAGGC-3'	(SEQ ID NO:88)
<u>reverse PCR primer</u> (45639.r2):	5'-CCTCCAGTAGGTGGATGGATTGGCTC-3'	(SEQ ID NO:89)

hybridization probe (45639.p1):

(SEQ ID NO:90)

5'-CTCACCTCATGAGGATGAGGCCATGGTGCTATTCCTCAACATGGTAG-3'

Clone DNA61185-1646 (Fig. 43, SEQ ID NO:84) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 130-132 and ending at the stop codon (TGA) at nucleotide positions 2110-2112 (Figure 43), as indicated by bolded underline. The predicted PRO1475 polypeptide precursor of Figure 44 (SEQ ID NO:85) is 660 amino acids long. The PRO1475 protein (SEQ ID NO:85) shown in Figure 44 has an estimated molecular weight of 75,220 Daltons and a pI of about 6.76. A clone containing DNA61185-1646 (SEQ ID NO:84) has been deposited with the ATCC on November 17, 1998 (under the designation DNA61185-1646) and has been assigned ATCC deposit number 203464.

Analysis of the PRO1475 protein sequence of Figure 44 (SEQ ID NO:85) reveals a transmembrane domain at about amino acid residues 38-55, N-myristoylation sites at about residues 276-282, 309-315, 505-511, 606-612, amidation site at about residues 213-217 and a region homologous region to Mouse GNT1 at about residues 229-660.

#### W. Isolation of cDNA clones Encoding Human PRO1271 (UNQ641)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence 312. This sequence was then compared to a variety of various EST as described under the signal algorithm procedure above, and further resulted in the identification of the EST Merck AA625350. Further examination and sequence of the full-length clone corresponding to this EST sequence (clone 1047230) resulted in the isolation of the full-length DNA sequence DNA66309-1538-1 (Fig. 45, SEQ ID NO:91) and the derived PRO1271 native sequence protein of Figure 46 (SEQ ID NO:92).

The full-length clone shown in Figure 45 (DNA66309, SEQ ID NO:91) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 94-96 and ending at the stop codon (TAA) at nucleotide positions 718-720, as indicated by bolded underline. The predicted PRO1271 polypeptide precursor of Figure 46 (SEQ ID NO:92) is 208 amino acids long, has a calculated molecular weight of about 21,531 Daltons and an estimated pI of approximately 8.99. A cDNA clone containing DNA66309-1538-1 (SEQ ID NO:91) was deposited with the ATCC on September 15, 1998 and is assigned ATCC deposit No. 203235.

Additional analysis of the PRO1271 (SEQ ID NO:92) polypeptide reveals a signal peptide at amino acid residues 1 to about 31, a transmembrane domain at about residues 166-187, N-glycosylation sites at about residues 46-50, 50-54, 64-68, 68-72, 83-87, 96-100, 106-110, 124-128, 138-142 and N-myristoylation sites at about residues 4-10, 7-13, 42-48, 101-107, 167-173, 172-178.

#### X. Isolation of cDNA clones Encoding Human PRO1343 (UNQ698)

Use of the amylase yeast screen procedure described above on tissue isolated from human smooth muscle cell tissue resulted in an EST sequence which served as the template for the creation of the oligonucleotides below and screening as described above in a human smooth muscle cell tissue library resulted in the isolation of the full length DNA sequence DNA66675-1587 (Fig. 47, SEQ ID NO:93) and the derived PRO1343 native sequence protein of Figure 48 (SEQ ID NO:94).

The oligonucleotide probes employed were as follows:

forward PCR primer (48921.f1): 5'-CAATATGCATCTTGCACGTCTGG-3' (SEQ ID NO:95)

reverse PCR primer (48921.r1): 5'-AAGCTTCTCTGCTTCCTTTCCTGC-3' (SEQ ID NO:96)

hybridization probe (48921.pl):

5'-TGACCCCATTTGAGAAGGTCATTGAAGGGATCAACCGAGGGCTG-3' (SEQ ID NO:97)

The full length clone DNA66675-1587 (SEQ ID NO:93) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 71-73, and a stop signal (TAA) at nucleotide positions 812-814 (Figure 47), as indicated by bolded underline. The predicted PRO1343 polypeptide precursor of Figure 48 (SEQ ID NO:94) is 247 amino acids long, has a calculated molecular weight of approximately 25,335 daltons and an estimated pI of approximately 7.0.

Further analysis of the PRO1343 sequence shown in Figure 48 (SEQ ID NO:94) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 25, N-myristoylation sites at residues 17-23, 35-41, 39-45, 53-59, 57-63, 76-82, 89-95, 104-110, 118-124, 140-146, 152-158, 154-160, 172-178, 190-196, 204-210, 215-221, 225-231 and a homologous region to circumsporozoite repeats from about amino acid 35 to about amino acid 225. A cDNA clone containing DNA66675-1587 (SEQ ID NO:93), has been deposited with ATCC on September 22, 1998 and is assigned ATCC deposit no. 203282.

Alternatively, a comparison of the yeast EST sequence isolated from the amylase screen above was screened against various EST databases, both public and private (e.g., see ECD homology procedure, above) resulting in the identification of Incyte EST clone no. 4701148. Further analysis and sequencing of the corresponding full-length clone resulted in isolation of the DNA66675-1587 sequence (SEQ ID NO:93) shown in Figure 47.

#### Y. Isolation of cDNA clones Encoding Human PRO1375 (UNQ712)

Use of the ECD homology procedure describe above in a human pancreas library resulted in the identification of the GenBank sequence AA143093. The corresponding full-length clone was examined and sequenced resulting in the isolation of the full-length DNA sequence DNA67004-1614 (Fig. 49, SEQ ID NO:98) and the derived PRO1375 native sequence protein of Figure 50 (SEQ ID NO:99).

Clone DNA67004-1614 (SEQ ID NO:98) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 104-106 and ending at the stop codon (TAA) at nucleotide positions 698-700 (Figure 49), as indicated by bolded underline. The predicted PRO1375 polypeptide precursor of Figure 49 (SEQ ID NO:99) is 198 amino acids long. The PRO1375 protein (SEQ ID NO:99) shown in Figure 50 has an estimated molecular weight of 22,531 and a pI of about 8.47. A cDNA clone containing DNA67004-1614 (SEQ ID NO:98) has been deposited with the ATCC on August 11, 1998 under the designation DNA67004-1614 and is assigned deposit number 203115. In the event of a discrepancy between the sequence as disclosed herein and the sequence of the deposit, it is understood that the deposited clone contains the correct sequence, and the sequences provided herein were produced using known sequencing techniques.

Analysis of the amino acid sequence of PRO1375 polypeptide (SEQ ID NO:99) reveals transmembrane domains at about amino acid residues 11-28 (type II) and at about residues 103-125, N-glycosylation site at about residues 60-64, a tyrosine kinase phosphorylation site at about residues 78-86 and an N-myristoylation site at about residues 12-18.

#### Z. Isolation of cDNA clones Encoding Human PRO1418 (UNQ732)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence 10698 (Incyte cluster 121480). This sequence was then compared to a variety of various EST databases (including those derived from a placenta tissue library) as described under the signal algorithm

procedure above, and further resulted in the identification of Incyte EST1306026. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA68864-1629 (Fig. 51, SEQ ID NO:100) and the derived PRO1418 native sequence protein of Figure 52 (SEQ ID NO:101).

- 5       The full length clone shown in Figure 51 (DNA68864-1629, SEQ ID NO:100) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 138-140 and ending at the stop codon (TAA) found at nucleotide positions 1188-1190, as indicated by bolded underline. The predicted PRO1418 polypeptide precursor of Figure 52 (SEQ ID NO:101) is 350 amino acids long with a signal peptide at about amino acids 1-19, a calculated molecular weight of approximately 39003 daltons, an estimated pI of approximately 5.59, N-glycosylation sites at about amino acids 128-132, N-myristoylation sites at about residues 189-195 and an amidation site at about residues 110-114. A cDNA clone containing DNA68864-1629 (SEQ ID NO:184) was deposited with the ATCC on September 22, 1998 and is assigned ATCC deposit no. 203276.

AA. Isolation of cDNA clones Encoding Human PRO1474 (UNO745)

- 15       Use of the ECD homology procedure described above resulted in the identification of the Incyte EST sequence 1843692. Further analysis and sequencing of the corresponding full-length clone resulted in the isolation of the full-length DNA sequence DNA73739-1645 (Fig. 53, SEQ ID NO:102) and the derived PRO1474 native sequence protein of Figure 54 (SEQ ID NO:103).

- 20       The full-length clone DNA73739-1645 (SEQ ID NO:102) shown in Figure 53 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 45-47 and a stop codon (TAA) at nucleotide positions 300-302, as indicated by bolded underline. The predicted PRO1474 polypeptide precursor of Figure 54 (SEQ ID NO:103) is 85 amino acids long, has a calculated molecular weight of 9232 Daltons and has a pI of about 7.94. A cDNA containing DNA73739-1645 (SEQ ID NO:102) has been deposited with the ATCC under the designation DNA73739-1645 on September 22, 1998 and is assigned ATCC deposit No. 203270.

Further analysis of the PRO1474 polypeptide (SEQ ID NO:103) of Figure 53 reveals a signal peptide at about amino acids residues 1-19, a kazal serine protease inhibitor family signature at about residues 45-68, a tyrosine kinase phosphorylation site at about residues 28-36 and an integrin alpha chain protein domain at about residues 32-42.

- 30   AB. Isolation of cDNA clones Encoding Human PRO1917 (UNO900)

- Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence 85496. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST3255033. This EST was derived from an ovarian tumor library. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA76400-2528 (Fig. 55, SEQ ID NO:104) and the derived PRO1917 native sequence protein of Figure 56 (SEQ ID NO:105).

- 40       The full length clone DNA76400-2528 (SEQ ID NO:104) shown in Figure 55 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 6 to 9 and ending at the stop codon (TGA) found at nucleotide positions 1467 to 1469 as indicated by bolded underline. The predicted PRO1917 polypeptide precursor of Figure 56 (SEQ ID NO:105) is 487 amino acids long, has a calculated

molecular weight of approximately 55,051 daltons and an estimated pI of approximately 8.14. Additional features include: a signal peptide at about amino acid residues 1-30; potential N-glycosylation sites at about amino acid residues 242 and 481, protein kinase C phosphorylation sites at about amino acid residues 95-97, 182-184, and 427-429; N-myristoylation sites at about amino acid residues 107-112, 113-118, 117-122, 118-123, and 128-133; and an endoplasmic reticulum targeting sequence at about amino acid residues 484-487. A cDNA clone containing DNA76400-2528 (SEQ ID NO:104) has been deposited with the ATCC on January 12, 1999 and is assigned ATCC deposit No. 203573.

AC. Isolation of cDNA clones Encoding Human PRO5723 (1972)

The cDNA DNA82361 (Figure 57, SEQ ID NO:106) which encodes the PRO5723 protein of Figure 58 (SEQ ID NO:107) is publicly available as GenBank accession number P78310 and is also described in Bergelson, J.M. *et al.*, *Science* **275**: 1320-1323 (1997), Tomko, R.P. *et al.*, *Proc. Natl. Acad. Sci. USA* **94**: 3352-3356 (1997) and Bowles, K.R. *et al.*, *Hum. Genet.* **105**: 354-359 (1999). The sequence is alternatively known as human coxsackievirus and adenovirus receptor precursor.

The entire nucleotide sequence of DNA82361 is shown in Figure 57 (SEQ ID NO:106). Clone DNA82361 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 71-73 and ending at the stop codon (TAA) at positions 1133-1135 (Fig. 57, SEQ ID NO:106), as indicated by bolded underline. The predicted PRO5723 polypeptide precursor of Fig. 58 (SEQ ID NO:107) is 352 amino acids in length, has a calculated molecular weight 38938 and a pI of 7.86.

Additional analysis of the PRO5723 polypeptide of Figure 58 (SEQ ID NO:107) reveals the presence of: a signal sequence at about amino acid residues 1-19, a transmembrane domain at about amino acid residues 235-256, N-glycosylation sites at about amino acid residues 106-110, 201-205, 298-302, tyrosine kinase phosphorylation sites at about residues 31-39, 78-85, 263-270, N-myristoylation sites at about residues 116-122, 208-214, 219-225, 237-243, 241-247, 245-251, 296-302, a myelin P0 protein at about residues 96-125, 229-283 and an immunoglobulin domain at about residues 34-122 and 155-214.

AD. Isolation of cDNA clones Encoding Human PRO4405 (UNQ1930)

Use of the ECD homology procedure described above in a human fetal kidney library resulted in the isolation of the full-length DNA sequence DNA84920-2614 (Fig. 59, SEQ ID NO:108) and the derived PRO4405 native sequence protein of Figure 60, SEQ ID NO:109).

The PCR primers (forward and reverse) and hybridization probe synthesized were:

forward PCR primer: 5'-CGGGACTTTCGCTACCTGTTGC-3' (SEQ ID NO:110)

reverse PCR primer: 5'-CATCATATTCCACAAAATGCTTTGGG-3' (SEQ ID NO:111)

hybridization probe: 5'-CCTTCGGGGATTCTTCCCGGCTCCCGTTCGTTCTCTCTG-3' (SEQ ID NO:112)

Clone DNA84920-2614 (Fig. 59, SEQ ID NO:108) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 79-81 and ending at the stop codon (TAG) at nucleotide positions 1009-1011 (Figure 59), as indicated by bolded underline. The predicted PRO4405 polypeptide precursor of Figure 60 (SEQ ID NO:109) is 310 amino acids long, has an estimated molecular weight of 33,875 Daltons and a pI of about 7.08. A clone containing DNA84920-2614 (SEQ ID NO:108) has been deposited with the ATCC on April 27, 1999 and has been assigned ATCC deposit number 203966.

Further analysis of the PRO4405 polypeptide of Figure 60 (SEQ ID NO:109) reveals a signal peptide at amino acid residues 1 to about 34, a transmembrane domain at about residues 58-76, N-glycosylation sites at about residues 56-60 and 194-198, N-myristoylation sites at about residues 6-12, 52-58, 100-106, 125-131, 233-239, 270-276, 275-281, 278-284, an amidation site at residues 154-158 and a cell attachment sequence at residues 205-208.

AE. Isolation of cDNA clones Encoding Human PRO4302 (UNQ1866)

Use of the amylase screen procedure described above on tissue isolated from human tissue resulted in an EST sequence which was then compared against various EST databases to create a consensus sequence by a methodology as described above under the amylase yeast screen procedure and/or the ECD homology procedure. Further analysis of this consensus sequence resulted in the identification of Incyte EST no. 2408081H1. Analysis of the full-length clones corresponding to EST no. 2408081H1 resulted in the isolation of the full length native sequence clones DNA92218-2554 (Fig. 61, SEQ ID NO:113) and the derived PRO4302 full-length native sequence protein of Figure 62 (SEQ ID NO:114).

The full length clone DNA92218-2554 (SEQ ID NO:113) shown in Figure 61 has a single open reading frame with an apparent translational initiation site at nucleotide positions 174-176 and a stop signal (TAG) at nucleotide positions 768-770, as indicated by bolded underline. The predicted PRO4302 polypeptide precursor of Figure 62 (SEQ ID NO:114) is 198 amino acids long, has a calculated molecular weight of approximately 22,285 daltons and an estimated pI of approximately 9.35. Analysis of PRO4302 of Figure 62 (SEQ ID NO:114) reveals a signal peptide from about amino acid residue 1 to about residue 23, a transmembrane domain from about amino acid residue 111 to about residue 130, a cAMP and cGMP-dependent protein kinase phosphorylation sites at residues 26-30, casein kinase II phosphorylation sites at residues 44-47 and 58-61, a tyrosine kinase phosphorylation site at residues 36-43 and N-myristoylation sites at residues 124-130, 144-150 and 189-195.

A cDNA clone containing DNA92218-2554 (SEQ ID NO:113) was deposited with the ATCC on March 9, 1999 and has been assigned deposit number 203834.

AF. Isolation of cDNA clones Encoding Human PRO9940 (UNQ889)

The cDNA DNA92282 (Figure 63, SEQ ID NO:115) which encodes the PRO9940 protein of Figure 63 (SEQ ID NO:115) is publicly available as GenBank accession number NM\_013371 or AF192498. The sequence is alternatively known as human IL-19.

The entire nucleotide sequence of DNA92282 (SEQ ID NO:115) is shown in Figure 63 (SEQ ID NO:115). Clone DNA92282 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 33-35 and ending at the stop codon (TGA) at positions 564-566 (Fig. 63, SEQ ID NO:115), as indicated by bolded underline. The predicted PRO9940 polypeptide precursor of Fig. 64 (SEQ ID NO:116) is 177 amino acids in length, has a calculated molecular weight of 20,452 and a pI of 8.00.

Additional analysis of the PRO9940 polypeptide of Figure 64 (SEQ ID NO:116) reveals the presence of: a signal sequence at about amino acid residues 1-18, N-glycosylation sites at about residues 56-60, 135-139, cAMP- and cGMP-dependent protein kinase phosphorylation site at about residues 102-106, N-myristoylation site at about residues 24-30 and an actinin-type actin-binding domain signature 1 at about residues 159-169.

AG. Isolation of cDNA clones Encoding Human PRO6006 (UNQ2516)

Use of the ECD homology procedure described above resulted in the isolation of the full-length DNA sequence DNA105782-2693 (Fig. 65, SEQ ID NO:117) and the derived PRO6006 native sequence protein of Figure 66 (SEQ ID NO:118).

5 The PCR primer (forward and reverse) and hybridization probe synthesized were:

forward PCR primer (43028.f1): 5'-TGAGCAGGAGTCACAGCACGAAGAC-3' (SEQ ID NO:119)

reverse PCR primer (43028.r1): 5'-TGAGTTGCATGCTTGAGGGCTGG-3' (SEQ ID NO:120)

hybridization probe (43028.p1): (SEQ ID NO:121)

5'-CTCCATCCTGACTGCTCCTCCTAAGAGAGATGGCACCGGCCAGAGCAGGATT-3'

10 Clone DNA105782-2693 (Fig. 65, SEQ ID NO:117) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 100-102 and ending at the stop codon (TAG) at nucleotide positions 568-570 (Figure 65), as indicated by bolded underline. The predicted PRO6006 polypeptide precursor shown in Figure 66 (SEQ ID NO:118) is 156 amino acids long, has a calculated molecular weight of 17,472 Daltons and a pI of 10.01. A clone containing DNA105782-2693 (SEQ ID  
15 NO:117) has been deposited with the ATCC on July 20, 1999 and has been assigned ATCC deposit number 387-PTA.

Further analysis of the PRO6006 polypeptide of Figure 66 (SEQ ID NO:118) reveals a signal peptide at about amino acid residues 1 to 22, N-glycosylation sites at about residues 127-131, cAMP- and cGMP-  
dependent protein kinase phosphorylation site at about residues 139-143, N-myristoylation sites at about  
20 residues 18-24, 32-38 and pancreatic ribonuclease family signature domains at about residues 65-72 and 49-93.

## EXAMPLE 2

### Stimulatory Activity in Mixed Lymphocyte Reaction (MLR) Assay (no.24)

This example shows that the polypeptides of the invention are active as a stimulator of the proliferation  
25 of stimulated T-lymphocytes. Compounds which stimulate proliferation of lymphocytes are useful therapeutically where enhancement of an immune response is beneficial. A therapeutic agent may take the form of antagonists of the polypeptide of the invention, for example, murine-human chimeric, humanized or human antibodies against the polypeptide.

The basic protocol for this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by  
30 J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum  
35 and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO<sub>2</sub>) and then washed and resuspended to 3 x 10<sup>6</sup> cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate).

The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads). The assay is prepared by plating in triplicate wells a mixture of: 100µl of test sample diluted to 1% or to 0.1%; 50 µl of irradiated  
40 stimulator cells and 50 µl of responder PBMC cells. 100 microliters of cell culture media or 100 microliter of



CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO<sub>2</sub> for 4 days. On day 5 and each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

- In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice.
- 5 The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to  $1 \times 10^7$  cells/ml of assay media. The assay is then conducted as described above. The results of this
- 10 assay for compounds of the invention are shown below. Positive increases over control are considered positive, with increases of greater than or equal to 180% being preferred. However, any value greater than control indicates a stimulatory effect for the test protein.

Table 7

	<u>PRO Concentration</u>	<u>Percent Increase Over Control</u>
15 PRO		
PRO861	95 nM	247.2
PRO861	9.5 nM	170.5
PRO788	29 nM	189.9
PRO788	2.9 nM	126
20 PRO1159	110.55 nM	186.6
PRO1159	11.06 nM	103.1
PRO1475	0.07 nM	137.5
PRO1475	0.7 nM	243
PRO1917	2.15 nM	115.2
25 PRO1917	21.46 nM	196.3
PRO9940	80.15 nM	183.3
PRO9940	8.02 nM	123
PRO9940	8.02 nM	170
PRO9940	80.15 nM	244.6
30 PRO9940	2.15 nM	115.2
PRO9940	21.46 nM	196.3
PRO5723	66 nM	187.8
PRO5723	6.6 nM	83.3
PRO6006	26.6 nM	199.9
35 PRO6006	2.66 nM	138.2

## EXAMPLE 3

Inhibitory Activity in Mixed Lymphocyte Reaction (MLR) Assay (no. 67)

- This example shows that one or more of the PRO polypeptides are active as inhibitors of the
- 40 proliferation of stimulated T-lymphocytes. Compounds which inhibit proliferation of lymphocytes are useful

therapeutically where suppression of an immune response is beneficial.

The basic protocol for this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

- 5 More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO<sub>2</sub>) and then washed and resuspended to 3x10<sup>6</sup> cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate). The  
10 stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads).

The assay is prepared by plating in triplicate wells a mixture of:

100:1 of test sample diluted to 1% or to 0.1%,

50 :1 of irradiated stimulator cells, and

- 15 50 :1 of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO<sub>2</sub> for 4 days. On day 5, each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

- In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice.  
20 The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to 1x10<sup>7</sup> cells/ml of assay media. The assay is then conducted as described above.

- 25 Any decreases below control is considered to be a positive result for an inhibitory compound, with decreases of less than or equal to 80% being preferred. However, any value less than control indicates an inhibitory effect for the test protein.

Table 8

	<u>PRO</u>	<u>PRO Concentration</u>	<u>Percent Decrease Below Control</u>
30	PRO184	1.00 %	67.4
	PRO184	0.10 %	123.2
	PRO184	0.65 nM	52.8
	PRO184	6.50 nM	51.9
	PRO184	5.34 nM	0
35	PRO184	5.34 nM	37.3
	PRO184	5.34 nM	50.7
	PRO184	5.34 nM	60.7
	PRO184	5.34 nM	77.6
	PRO184	5.34 nM	82.7
40	PRO184	53.39 nM	0

	PRO184	53.39 nM	5.8
	PRO184	53.40 nM	5
	PRO184	53.40 nM	7.2
	PRO184	53.40 nM	10.9
5	PRO184	53.40 nM	14.1
	PRO306	1.64 nM	77.1
	PRO306	16.41 nM	63.5
	PRO779	4.2 nM	50.4
	PRO779	0.42 nM	56.4
10	PRO1271	0.56 nM	27.3
	PRO1271	5.6 nM	60.3
	PRO1375	21.5 nM	74.2
	PRO1375	215 nM	59.9
	PRO1474	64 nM	22.8
15	PRO1474	6.4 nM	21.5

## EXAMPLE 4

Inhibition of co-stimulation of CD4+ enriched lymphocytes (ASY121)

This assay shows that one or more of the PRO polypeptides are active as inhibitors of the stimulation of CD4+ enriched lymphocytes. Compounds which inhibit proliferation of lymphocytes are useful therapeutically where suppression of an inflammatory immune response is beneficial. This assay is a variation of the MLR assay above wherein the PRO polypeptide is examined for its inhibitory effect upon the co-stimulation of CD4+ enriched lymphocytes with both anti-CD3 and anti-CD28. The inhibition of the stimulatory effect of anti-CD3 and anti-CD28 on PBMCs is proposed to correlate with a general antiproliferative effect similar to the engagement of the TCR with a costimulatory signal.

The basic protocol for the isolation of PBMCs used in this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis. Cells are isolated and enriched using negative selection. If desired, the enriched cells are frozen in 90% fetal bovine serum and 10% DMSO. Frozen cells may be thawed overnight in assay media (37°C, 5% CO<sub>2</sub>) and then washed and resuspended to 1x10<sup>6</sup> cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate).

The assay is prepared by plating in triplicate wells a mixture of:

100 ul of test sample diluted to indicated concentration

100 ul of cells

50 ul of anti-CD3 (50 ng/ml, Amac 0178) and 50 ul anti-CD28 (100 ng/ml, Biodesign P42235M) is added to a 96 well plate for an overnight coat at 4°C prior to the addition of cells and test sample.

100 microliters of cell buffer control or 100 microliter of Hu-IgG is used as the control in place of the test sample.

The wells are then incubated at 37°C, 5% CO<sub>2</sub> for about 3 days. On day 4, each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours, the plate is harvested and then the uptake of the  
5 label is evaluated.

A result which shows an inhibitory effect (i.e., <sup>3</sup>H-thymidine incorporation) less than 70% of that observed in the control is considered to be a positive result.

In another variant of this assay, CD4<sup>+</sup> splenocytes are isolated from the spleens of Balb/c mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the  
10 splenocytes are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media, negative selection and resuspending the cells to 1x10<sup>7</sup> cells/ml of assay media. The assay is then conducted as described above.

15

Table 9

PRO	concentration	inhibition
PRO184	53.39 nM	+
PRO212	4.64 nM	++
PRO306	16.41 nM	+
20 PRO333	21.34 nM	+
PRO364	17 nM	+
PRO381	16.95 nM	+
PRO982	8.44 nM	+
PRO1068	144 nM	+
25 PRO1157	497.92 nM	+
PRO1343	45.29 nM	+
PRO4302	135.57 nM	+
PRO4405	0.5 nM	+

### 30 EXAMPLE 5

#### Stimulation of peripheral blood mononuclear cells (PBMCs) or CD4<sup>+</sup> cells with anti CD3 and PRO protein (ASY99)

This assay shows that one or more of the PRO polypeptides are active as enhancers of the stimulation of PBMCs or CD4<sup>+</sup> cells. CD4<sup>+</sup> cells are enriched by negative selection using MACs beads after LSM  
35 separation. The ability of the PRO polypeptide to replace anti-CD28 is examined to determine the stimulatory effect.

Anti-CD3 and anti-CD28 are known to stimulate PBMCs. The basic protocol for the isolation of PBMCs used in this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John  
40 Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis. If desired, the cells are enriched for CD4+ cells, then frozen in 90% fetal bovine serum and 10% DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO<sub>2</sub>) and then washed and resuspended to 0.5x10<sup>6</sup> cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate).

The assay is prepared by plating in triplicate wells a mixture of: 200 ul of cells after the overnight coat of anti CD3 and SPD1 protein.

50 ul of anti-CD3 (50 ng/ml, Amac 0178) and 50 ul of 1% of SPD1 protein are coated on a 96 well plate in PBS 4°C overnight. 50 ul Hu-IgG is used as the control in place of the SPD1 protein.

The wells are then incubated at 37°C, 5% CO<sub>2</sub> for about 3 days. On day 4, each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours the cells are harvested and then the uptake of the label is evaluated.

A result which indicates a stimulatory effect (i.e., [<sup>3</sup>H]-thymidine incorporation) greater than 200% of the control is considered to be positive stimulatory result.

In another variant of this assay, PBMCs or CD4 + splenocytes are isolated from the spleens of Balb/c mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media. CD4+ cells are enriched by negative selection using beads, washed in media and resuspended the cells to 1x10<sup>7</sup> cells/ml of assay media. The assay is then conducted as described above.

Table 10

25	PRO	concentration	stimulation (+)/inhibition (-)
	PRO245	3.5 nM	+
	PRO266	1.8 nM	+
	PRO306	5.46 nM	+
	PRO333	9.2 nM	+
30	PRO356	1.1 nM	+
	PRO364	27.23 nM	+
	PRO381	145 nM	+
	PRO526	15.6 nM	+
	PRO719	1.07 nM	+
35	PRO719	1.07 nM	+
	PRO769	6.84 nM	+
	PRO826	9.03 nM	+
	PRO1031	5.6 nM	+
	PRO1069	16.72 nM	+
40	PRO1343	176 nM	+

PRO1343	176 nM	+
PRO1375	215 nM	+
PRO1418	63.98 nM	+

## EXAMPLE 6

In situ Hybridization

*In situ* hybridization is a powerful and versatile technique for the detection and localization of nucleic acid sequences within cell or tissue preparations. It may be useful, for example, to identify sites of gene expression, analyze the tissue distribution of transcription, identify and localize viral infection, follow changes in specific mRNA synthesis and aid in chromosome mapping.

*In situ* hybridization was performed following an optimized version of the protocol by Lu and Gillett, *Cell Vision* 1: 169-176 (1994), using PCR-generated <sup>33</sup>P-labeled riboprobes. Briefly, formalin-fixed, paraffin-embedded human tissues were sectioned, deparaffinized, deproteinized in proteinase K (20 g/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett, *supra*. A [<sup>33</sup>P] UTP-labeled antisense riboprobe was generated from a PCR product and hybridized at 55°C overnight. The slides were dipped in Kodak NTB2 nuclear track emulsion and exposed for 4 weeks.

<sup>33</sup>P-Riboprobe synthesis

6.0 µl (125 mCi) of <sup>33</sup>P-UTP (Amersham BF 1002, SA<2000 Ci/mmol) were speed vac dried. To each tube containing dried <sup>33</sup>P-UTP, the following ingredients were added: 2.0 µl 5x transcription buffer; 1.0 µl DTT (100 mM); 2.0 µl NTP mix (2.5 mM : 10 µl; each of 10 mM GTP, CTP & ATP + 10 µl H<sub>2</sub>O); 1.0 µl UTP (50 µM); 1.0 µl Rnasin; 1.0 µl DNA template (1µg); 1.0 µl H<sub>2</sub>O.

The tubes were incubated at 37°C for one hour. 1.0 µL RQ1 DNase were added, followed by incubation at 37°C for 15 minutes. 90 µL TE (10 mM Tris pH 7.6/1mM EDTA pH 8.0) were added, and the mixture was pipetted onto DE81 paper. The remaining solution was loaded in a Microcon-50 ultrafiltration unit, and spun using program 10 (6 minutes). The filtration unit was inverted over a second tube and spun using program 2 (3 minutes). After the final recovery spin, 100 µL TE were added. 1 µL of the final product was pipetted on DE81 paper and counted in 6 ml of Biofluor II.

The probe was run on a TBE/urea gel. 1-3 µL of the probe or 5 µL of RNA Mrk III were added to 3 µL of loading buffer. After heating on a 95°C heat block for three minutes, the gel was immediately placed on ice. The wells of gel were flushed, the sample loaded, and run at 180-250 volts for 45 minutes. The gel was wrapped in saran wrap and exposed to XAR film with an intensifying screen in -70°C freezer one hour to overnight.

<sup>33</sup>P-Hybridization

*Pretreatment of frozen sections* The slides were removed from the freezer, placed on aluminum trays and thawed at room temperature for 5 minutes. The trays were placed in 55°C incubator for five minutes to reduce condensation. The slides were fixed for 10 minutes in 4% paraformaldehyde on ice in the fume hood, and washed in 0.5 x SSC for 5 minutes, at room temperature (25 ml 20 x SSC + 975 ml SQ H<sub>2</sub>O). After deproteinization in 0.5 µg/ml proteinase K for 10 minutes at 37°C (12.5µL of 10 mg/ml stock in 250 ml

prewarmed RNase-free RNase buffer), the sections were washed in 0.5 x SSC for 10 minutes at room temperature. The sections were dehydrated in 70%, 95%, 100% ethanol, 2 minutes each.

*Pretreatment of paraffin-embedded sections* The slides were deparaffinized, placed in SQ H<sub>2</sub>O, and rinsed twice in 2 x SSC at room temperature, for 5 minutes each time. The sections were deproteinized in 20 µg/ml proteinase K (500 µL of 10 mg/ml in 250 ml RNase-free RNase buffer; 37°C, 15 minutes) - human embryo, or 8 x proteinase K (100 µL in 250 ml RNase buffer, 37°C, 30 minutes) - formalin tissues. Subsequent rinsing in 0.5 x SSC and dehydration were performed as described above.

*Prehybridization* The slides were laid out in plastic box lined with Box buffer (4 x SSC, 50% formamide) - saturated filter paper. The tissue was covered with 50 µL of hybridization buffer (3.75g Dextran Sulfate + 6 ml SQ H<sub>2</sub>O), vortexed and heated in the microwave for 2 minutes with the cap loosened. After cooling on ice, 18.75 ml formamide, 3.75 ml 20 x SSC and 9 ml SQ H<sub>2</sub>O were added, the tissue was vortexed well, and incubated at 42°C for 1-4 hours.

*Hybridization* 1.0 x 10<sup>6</sup> cp. probe and 1.0 µL RNA (50 mg/ml stock) per slide were heated at 95°C for 3 minutes. The slides were cooled on ice, and 48 µL hybridization buffer were added per slide. After vortexing, 50 µL <sup>33</sup>P mix were added to 50 µL prehybridization on slide. The slides were incubated overnight at 55°C.

*Washes* Washing was done 2x10 minutes with 2xSSC, EDTA at room temperature (400 ml 20 x SSC + 16 ml 0.25M EDTA, V<sub>f</sub>=4L), followed by RNaseA treatment at 37°C for 30 minutes (500 µL of 10 mg/ml in 250 ml RNase buffer - 20 µg/ml). The slides were washed 2x10 minutes with 2 x SSC, EDTA at room temperature. The stringency wash conditions were as follows: 2 hours at 55°C, 0.1 x SSC, EDTA (20 ml 20 x SSC + 16 ml EDTA, V<sub>f</sub>=4L).

Alternatively, multi-tissue blots containing poly A<sup>+</sup> RNA (2 µg per lane) from various human tissues were purchased from Clontech (Palo Alto, CA). DNA probes were labeled with [α-<sup>32</sup>P]dCTP by random priming DNA labeling Beads (Pharmacia Biotech). Hybridization was performed with Expresshyb (Clontech) at 68°C for 1 hr. The blots were then washed with 2X SSC/0.05% SDS solution at room temperature for 40 min, followed by washes in 0.1X SSC/0.1%SDS solution at 55°C for 40 min with one change of fresh solution. The blots were exposed in a phosphorimager.

## EXAMPLE 7

### In situ Hybridization in Cells and Diseased Tissues

The *in situ* hybridization method of Example 6 is used to determine gene expression, analyze the tissue distribution of transcription, and follow changes in specific mRNA synthesis for the genes/DNAs and the proteins of the invention in diseased tissues isolated from human individuals suffering from a specific disease. These results show more specifically where in diseased tissues the genes of the invention are expressed and are more predictive of the particular localization of the therapeutic effect of the inhibitory or stimulatory compounds of the invention (and agonists or antagonists thereof) in a disease. Hybridization is performed according to the method of Example 6 using one or more of the following tissue and cell samples:

(a) lymphocytes and antigen presenting cells (dendritic cells, Langherhans cells, macrophages and monocytes, NK cells);

(b) lymphoid tissues: normal and reactive lymph node, thymus, Bronchial Associated Lymphoid Tissues, (BALT), Mucosal Associated Lymphoid Tissues (MALT);

5 (c) human disease tissues:

- Synovium and joint of patients with Arthritis and Degenerative Joint Disease;
- Colon from patients with Inflammatory Bowel Disease including Ulcerative Colitis and Crohns' disease;
- Skin lesions from Psoriasis and other forms of dermatitis;
- 10 • Lung tissue including BALT and tissue lymph nodes from chronic and acute bronchitis, pneumonia, pneumonitis, pleuritis;
- Lung tissue including BALT and tissue lymph nodes from Asthma;
- nasal and sinus tissue from patients with rhinitis or sinusitis;
- Brain and Spinal cord from Multiple Sclerosis, Alzheimer's Disease and Stroke;
- 15 • Kidney from Nephritis, Glomerulonephritis and Systemic Lupus Erythematosus;
- Liver from Infectious and non-infectious Hepatitis and acetaminophen-induced liver cirrhosis;
- Tissues from Neoplasms/Cancer.

20 Expression is observed in one or more cell or tissue samples indicating localization of the therapeutic effect of the compounds of the invention (and agonists or antagonists thereof) in the disease associated with the cell or tissue sample.

The sequences of the oligonucleotides used, where expression overlaps with the non-diseased tissue distribution reported earlier is recited in Example 6.

25 DNA67004:

IS99-109:

DNA67004 (SEQ ID NO:98) has weak diffuse signal in a single section of tonsil. There was weak some signal in the mucosal epithelium in colitis samples and as well as chronic asthma. There was also focal expression in an area of hyperplastic epidermis in a one section of psoriatic skin.

30 The probes used for the above procedures were the following:

DNA67004.p1: (SEQ ID NO:122)

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC GGG AGA GGG GAG GGA TGC-3'

DNA67004.p2: (SEQ ID NO:123)

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GAC CGG AAA TGC TGA CAA ATG-3'

35

#### EXAMPLE 8

##### Use of the PRO polypeptides as a hybridization probe

The following method describes use of a nucleotide sequence encoding PRO as a hybridization probe.



DNA comprising the coding sequence of full-length or mature PRO as disclosed herein is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C. DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO can then be identified using standard techniques known in the art.

#### EXAMPLE 9

##### Expression of the PRO polypeptide in *E. coli*

This example illustrates preparation of an unglycosylated form of PRO by recombinant expression in *E. coli*.

The DNA sequence encoding PRO is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar *et al.*, *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a *trp* promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO coding region, lambda transcriptional terminator, and an *argU* gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook *et al.*, *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

PRO may be expressed in *E. coli* in a poly-His tagged form, using the following procedure. The DNA encoding PRO is initially amplified using selected PCR primers. The primers will contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-amplified, poly-His tagged sequences are then

ligated into an expression vector, which is used to transform an *E. coli* host based on strain 52 (W3110 fuhA(tonA) lon galE rpoHts(hrpRts) clpP(lacIq). Transformants are first grown in LB containing 50 mg/ml carbenicillin at 30 °C with shaking until an O.D.600 of 3-5 is reached. Cultures are then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.71 g sodium citrate-2H<sub>2</sub>O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g Sheffield hycase SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM MgSO<sub>4</sub>) and grown for approximately 20-30 hours at 30 °C with shaking. Samples are removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells. Cell pellets are frozen until purification and refolding.

*E. coli* paste from 0.5 to 1 L fermentations (6-10 g pellets) is resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution is stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution is centrifuged at 40,000 rpm in a Beckman Ultracentrifuge for 30 min. The supernatant is diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. The clarified extract is loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column is washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein is eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein are pooled and stored at 4°C. Protein concentration is estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins are refolded by diluting the sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes are chosen so that the final protein concentration is between 50 to 100 micrograms/ml. The refolding solution is stirred gently at 4°C for 12-36 hours. The refolding reaction is quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution is filtered through a 0.22 micron filter and acetonitrile is added to 2-10% final concentration. The refolded protein is chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance are analyzed on SDS polyacrylamide gels and fractions containing homogeneous refolded protein are pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded PRO polypeptide are pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins are formulated into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

## EXAMPLE 10

Expression of the PRO polypeptides in mammalian cells

This example illustrates preparation of a potentially glycosylated form of PRO by recombinant expression in mammalian cells.

5       The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO DNA using ligation methods such as described in Sambrook *et al.*, *supra*. The resulting vector is called pRK5-PRO.

10       In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg pRK5-PRO DNA is mixed with about 1 µg DNA encoding the VA RNA gene [Thimmappaya *et al.*, *Cell*, 31:543 (1982)] and dissolved in 500 µl of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl<sub>2</sub>. To this mixture is added, dropwise, 500 µl of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO<sub>4</sub>, and a precipitate is allowed to form for 10 minutes at 25°C. The  
15       precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

      Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 µCi/ml <sup>35</sup>S-cysteine and 200 µCi/ml <sup>35</sup>S-methionine.  
20       After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

      In an alternative technique, PRO may be introduced into 293 cells transiently using the dextran sulfate  
25       method described by Somparyrac *et al.*, *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5-PRO DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and  
30       0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

      In another embodiment, PRO can be expressed in CHO cells. The pRK5-PRO can be transfected into CHO cells using known reagents such as CaPO<sub>4</sub> or DEAE-dextran. As described above, the cell cultures can be  
35       incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as <sup>35</sup>S-methionine. After determining the presence of PRO polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO can then be concentrated and purified by any selected method.

Epitope-tagged PRO may also be expressed in host CHO cells. The PRO may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO can then be concentrated and purified by any selected method, such as by  $\text{Ni}^{2+}$ -chelate affinity chromatography.

PRO may also be expressed in CHO and/or COS cells by a transient expression procedure or in CHO cells by another stable expression procedure.

Stable expression in CHO cells is performed using the following procedure. The proteins are expressed as an IgG construct (immunoadhesin), in which the coding sequences for the soluble forms (e.g. extracellular domains) of the respective proteins are fused to an IgG1 constant region sequence containing the hinge, CH2 and CH2 domains and/or is a poly-His tagged form.

Following PCR amplification, the respective DNAs are subcloned in a CHO expression vector using standard techniques as described in Ausubel *et al.*, *Current Protocols of Molecular Biology*, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' and 3' of the DNA of interest to allow the convenient shuttling of cDNA's. The vector used expression in CHO cells is as described in Lucas *et al.*, *Nucl. Acids Res.* 24:9 (1774-1779 (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA is introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect<sup>®</sup> (Quiagen), Dosper<sup>®</sup> or Fugene<sup>®</sup> (Boehringer Mannheim). The cells are grown as described in Lucas *et al.*, *supra*. Approximately  $3 \times 10^7$  cells are frozen in an ampule for further growth and production as described below.

The ampules containing the plasmid DNA are thawed by placement into water bath and mixed by vortexing. The contents are pipetted into a centrifuge tube containing 10 mL of media and centrifuged at 1000 rpm for 5 minutes. The supernatant is aspirated and the cells are resuspended in 10 mL of selective media (0.2  $\mu\text{m}$  filtered PS20 with 5% 0.2  $\mu\text{m}$  diafiltered fetal bovine serum). The cells are then aliquoted into a 100 mL spinner containing 90 mL of selective media. After 1-2 days, the cells are transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, 250 mL, 500 mL and 2000 mL spinners are seeded with  $3 \times 10^5$  cells/mL. The cell media is exchanged with fresh media by centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in U.S. Patent No. 5,122,469, issued June 16, 1992 may actually be used. A 3L production spinner is seeded at  $1.2 \times 10^6$  cells/mL. On day 0, the cell number pH is determined. On day 1, the spinner is sampled and sparging with filtered air is commenced. On day 2, the spinner is sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (e.g., 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion) taken. Throughout the production, the pH is adjusted as necessary to keep it at around 7.2. After 10 days, or until the viability dropped below 70%, the cell culture is harvested by centrifugation and filtering through a 0.22  $\mu\text{m}$  filter. The filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins are purified using a Ni-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc-containing) constructs are purified from the conditioned media as follows. The conditioned medium is pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275  $\mu$ L of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity is assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

#### EXAMPLE 11

##### Expression of PRO in Yeast

The following method describes recombinant expression of PRO in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of PRO from the ADH2/GAPDH promoter. DNA encoding PRO and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of PRO. For secretion, DNA encoding PRO can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of PRO.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO may further be purified using selected column chromatography resins.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

#### EXAMPLE 12

##### Expression of PRO in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of PRO in Baculovirus-infected insect cells.

The sequence coding for PRO is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A

variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO or the desired portion of the coding sequence of PRO such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley *et al.*, *Baculovirus expression vectors: A Laboratory Manual*, Oxford: Oxford University Press (1994).

Expressed poly-his tagged PRO can then be purified, for example, by Ni<sup>2+</sup>-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert *et al.*, *Nature*, **362**:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl<sub>2</sub>; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.3) and filtered through a 0.45 µm filter. A Ni<sup>2+</sup>-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A<sub>280</sub> with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A<sub>280</sub> baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni<sup>2+</sup>-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His<sub>10</sub>-tagged PRO are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) PRO can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

### EXAMPLE 13

#### Preparation of Antibodies that Bind PRO

This example illustrates preparation of monoclonal antibodies which can specifically bind PRO.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified PRO, fusion proteins containing PRO, and cells expressing recombinant PRO on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and

injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO antibodies.

5 After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and  
10 thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against PRO. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO monoclonal antibodies. Alternatively, the hybridoma cells can be grown in  
15 tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

#### EXAMPLE 14

##### 20 Purification of PRO Polypeptides Using Specific Antibodies

Native or recombinant PRO polypeptides may be purified by a variety of standard techniques in the art of protein purification. For example, pro-PRO polypeptide, mature PRO polypeptide, or pre-PRO polypeptide is purified by immunoaffinity chromatography using antibodies specific for the PRO polypeptide of interest. In general, an immunoaffinity column is constructed by covalently coupling the anti-PRO polypeptide antibody to  
25 an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a  
30 chromatographic resin such as CnBr-activated SEPHAROSE™ (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such an immunoaffinity column is utilized in the purification of PRO polypeptide by preparing a fraction from cells containing PRO polypeptide in a soluble form. This preparation is derived by solubilization  
35 of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble PRO polypeptide containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble PRO polypeptide-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PRO polypeptide (e.g., high ionic  
40 strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt

antibody/PRO polypeptide binding (e.g., a low pH buffer such as approximately pH 2-3, or a high concentration of a chaotrope such as urea or thiocyanate ion), and PRO polypeptide is collected.

#### EXAMPLE 15

##### Drug Screening

This invention is particularly useful for screening compounds by using PRO polypeptides or binding fragment thereof in any of a variety of drug screening techniques. The PRO polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the PRO polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between PRO polypeptide or a fragment and the agent being tested. Alternatively, one can examine the diminution in complex formation between the PRO polypeptide and its target cell or target receptors caused by the agent being tested.

Thus, the present invention provides methods of screening for drugs or any other agents which can affect a PRO polypeptide-associated disease or disorder. These methods comprise contacting such an agent with an PRO polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the PRO polypeptide or fragment, or (ii) for the presence of a complex between the PRO polypeptide or fragment and the cell, by methods well known in the art. In such competitive binding assays, the PRO polypeptide or fragment is typically labeled. After suitable incubation, free PRO polypeptide or fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to PRO polypeptide or to interfere with the PRO polypeptide/cell complex.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to a polypeptide and is described in detail in WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. As applied to a PRO polypeptide, the peptide test compounds are reacted with PRO polypeptide and washed. Bound PRO polypeptide is detected by methods well known in the art. Purified PRO polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding PRO polypeptide specifically compete with a test compound for binding to PRO polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRO polypeptide.

#### EXAMPLE 15

##### Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptide of interest (i.e., a PRO polypeptide) or of small molecules with which they interact, e.g., agonists, antagonists, or inhibitors. Any of these examples can be used to fashion drugs which are more active or stable forms of the



PRO polypeptide or which enhance or interfere with the function of the PRO polypeptide *in vivo* (c.f., Hodgson, *BioTechnology*, 9: 19-21 (1991)).

5 In one approach, the three-dimensional structure of the PRO polypeptide, or of an PRO polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the PRO polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of the PRO polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous PRO polypeptide-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton and Wells, *Biochemistry*, 31:7796-7801 (1992) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda et al., *J. Biochem.*, 113:742-746 (1993).  
10

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.  
15

20 By virtue of the present invention, sufficient amounts of the PRO polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the PRO polypeptide amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

	<u>Material</u>	<u>UNQ</u>	<u>PRO</u>	<u>ATCC #</u>	<u>ATCC Deposit Date</u>
	DNA30942-1134	186	212	209254	September 16, 1997
	DNA35638-1141	219	245	209265	September 16, 1997
	DNA37150-1178	233	266	209401	October 17, 1997
5	DNA39984-1221	269	306	209435	November 7, 1997
	DNA41374-1312	294	333	-----	-----
	DNA44184-1319	330	526	209704	March 26, 1998
	DNA44194-1317	322	381	209808	April 28, 1998
	DNA47365-1206	319	364	209436	November 7, 1997
10	DNA47470-1130	313	356	209422	November 28, 1997
	DNA49646-1327	387	719	209705	March 26, 1998
	DNA54231-1366	407	769	209804	April 23, 1998
	DNA56405-1357	430	788	209849	May 6, 1998
	DNA57694-1341	467	826	203017	June 23, 1998
15	DNA57700-1408	483	982	203583	January 12, 1999
	DNA58801-1052	455	779	55820	July 5, 1996
	DNA59214-1449	525	1068	203046	July 1, 1998
	DNA59294-1381	516	1031	209866	May 14, 1998
	DNA60292-1506	587	1157	203540	December 15, 1998
20	DNA60627-1508	589	1159	203092	August 4, 1998
	DNA61185-1646	746	1475	203464	November 17, 1998
	DNA66309-1538-1	641	1271	203235	September 15, 1998
	DNA66675-1587	698	1343	203282	September 22, 1998
	DNA67004-1614	712	1375	203115	August 11, 1998
25	DNA68864-1629	732	1418	203276	September 22, 1998
	DNA73739-1645	745	1474	203270	September 22, 1998
	DNA76400-2528	900	1917	203573	January 12, 1999
	DNA84920-2614	1930	4405	203966	April 27, 1999
	DNA92218-2554	1866	4302	203834	March 9, 1999
30	DNA105782-2693	2516	6006	PTA-387	July 20, 1999

These deposits was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement

between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC  
5 122 and the Commissioner's rules pursuant thereto (including 37 CFR 1.14 with particular reference to 386 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to  
10 practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited; since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs  
15 that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description  
20 and fall within the scope of the appended claims.

What is claimed:

1. A composition useful for the treatment of immune related diseases, comprising a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, agonist or fragment thereof and a carrier or excipient, having the properties of:
  - (a) increasing infiltration of inflammatory cells into a tissue of a mammal in need thereof,
  - (b) stimulating or enhancing an immune response in a mammal in need thereof,
  - (c) increasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen, or
  - (d) stimulating the activity of T-lymphocytes in a mammal in need thereof in response to an antigen.
2. The composition of claim 1 comprising an effective amount of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, agonist, antagonist or fragment thereof.
3. The composition of claim 2 further comprising a growth inhibitory agent, cytotoxic agent or chemotherapeutic agent.
4. Use of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, agonist or a fragment thereof to prepare a composition having the properties of:
  - (a) increasing infiltration of inflammatory cells into a tissue of a mammal in need thereof,
  - (b) stimulating or enhancing an immune response in a mammal in need thereof, or
  - (c) increasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen, or
  - (d) stimulating the activity of T-lymphocytes in a mammal in need thereof in response to an antigen.
5. The use of claims 4 comprising an effective amount of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, agonist, antagonist or fragment thereof.

6. The composition of claim 2 further comprising a growth inhibitory agent, cytotoxic agent or chemotherapeutic agent.

7. A composition useful for the treatment of immune related diseases, comprising a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, agonist or fragment thereof and a carrier or excipient, having the properties of:

- (a) decreasing infiltration of inflammatory cells into a tissue of a mammal in need thereof,
- (b) inhibiting or reducing an immune response in a mammal in need thereof,
- (c) decreasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen, or
- (d) decreasing the activity of T-lymphocytes in a mammal in need thereof in response to an antigen.

15

8. The composition of claim 2 comprising an effective amount of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, agonist, antagonist or fragment thereof.

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9. The composition of claim 8 further comprising a growth inhibitory agent, cytotoxic agent or chemotherapeutic agent.

10. Use of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, agonist or a fragment thereof to prepare a composition having the properties of:

- (a) decreasing infiltration of inflammatory cells into a tissue of a mammal in need thereof,
- (b) inhibiting or reducing an immune response in a mammal in need thereof, or
- (c) decreasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen, or
- (d) decreasing the activity of T-lymphocytes in a mammal in need thereof in response to an antigen.

35

11. The use of claim 10 comprising an effective amount of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006

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polypeptide, agonist, antagonist or fragment thereof.

12. The composition of claim 11 further comprising a growth inhibitory agent, cytotoxic agent or chemotherapeutic agent.

5

13. A method of treating an immune related disorder in a mammal in need thereof, comprising administering to the mammal an effective amount of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, 10 PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, an agonist thereof or an antagonist thereto.

14. The method of claim 13, wherein the disorder is selected from systemic lupus erythematosus, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis, 15 idiopathic inflammatory myopathies, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious, autoimmune chronic active hepatitis, primary biliary 20 cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation 25 associated diseases including graft rejection and graft-versus-host-disease.

15. The composition or use of any of the preceding claims, wherein the agonist or antagonist is a monoclonal antibody.

16. The composition or use of any of the preceding claims, wherein the agonist or antagonist is an antibody fragment or a single-chain antibody. 30

17. The composition or use of claims 15 or 16, wherein the antibody has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues.

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18. A method for determining the presence of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, comprising 40 exposing a cell suspected of containing the polypeptide to an anti-PRO184, anti-PRO212, anti-PRO245, anti-

PRO266, anti-PRO306, anti-PRO333, anti-PRO526, anti-PRO381, anti-PRO364, anti-PRO356, anti-PRO719, anti-PRO861, anti-PRO769, anti-PRO788, anti-PRO826, anti-PRO982, anti-PRO779, anti-PRO1068, anti-PRO1031, anti-PRO1157, anti-PRO1159, anti-PRO1475, anti-PRO1271, anti-PRO1343, anti-PRO1375, anti-PRO1418, anti-PRO1474, anti-PRO1917, anti-PRO5723, anti-PRO4405, anti-PRO4302, anti-PRO9940 or anti-PRO6006 antibody, respectively, and determining binding of the antibody to the cell.

19. A method of diagnosing an immune related disease in a mammal, comprising detecting the level of expression of a gene encoding a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control indicates the presence of immune related disease in the mammal from which the test tissue cells were obtained.

20. A method of diagnosing an immune related disease in a mammal, comprising (a) contacting an anti-PRO184, anti-PRO212, anti-PRO245, anti-PRO266, anti-PRO306, anti-PRO333, anti-PRO526, anti-PRO381, anti-PRO364, anti-PRO356, anti-PRO719, anti-PRO861, anti-PRO769, anti-PRO788, anti-PRO826, anti-PRO982, anti-PRO779, anti-PRO1068, anti-PRO1031, anti-PRO1157, anti-PRO1159, anti-PRO1475, anti-PRO1271, anti-PRO1343, anti-PRO1375, anti-PRO1418, anti-PRO1474, anti-PRO1917, anti-PRO5723, anti-PRO4405, anti-PRO4302, anti-PRO9940 or anti-PRO6006 antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and the polypeptide in the test sample; wherein the formation of a complex is indicative of the presence or absence of said disease.

21. An immune related disease diagnostic kit, comprising an anti-PRO184, anti-PRO212, anti-PRO245, anti-PRO266, anti-PRO306, anti-PRO333, anti-PRO526, anti-PRO381, anti-PRO364, anti-PRO356, anti-PRO719, anti-PRO861, anti-PRO769, anti-PRO788, anti-PRO826, anti-PRO982, anti-PRO779, anti-PRO1068, anti-PRO1031, anti-PRO1157, anti-PRO1159, anti-PRO1475, anti-PRO1271, anti-PRO1343, anti-PRO1375, anti-PRO1418, anti-PRO1474, anti-PRO1917, anti-PRO5723, anti-PRO4405, anti-PRO4302, anti-PRO9940, anti-PRO6006 antibody or fragment thereof and a carrier in suitable packaging.

22. The kit of claim 21, further comprising instructions for using the antibody to detect a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide.

23. An article of manufacture, comprising:

(a) a composition of matter comprising a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779,

PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide or agonist or antagonist thereof;

(b) a container containing said composition; and

5 (c) an instruction affixed to said container, or a package insert included in said container referring to the use of said PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide or agonist or antagonist thereof in the treatment of an immune  
10 related disease.

24. The article of manufacture of claim 23 wherein said active agent is an anti-PRO184, anti-PRO212, anti-PRO245, anti-PRO266, anti-PRO306, anti-PRO333, anti-PRO526, anti-PRO381, anti-PRO364, anti-PRO356, anti-PRO719, anti-PRO861, anti-PRO769, anti-PRO788, anti-PRO826, anti-PRO982, anti-  
15 PRO779, anti-PRO1068, anti-PRO1031, anti-PRO1157, anti-PRO1159, anti-PRO1475, anti-PRO1271, anti-PRO1343, anti-PRO1375, anti-PRO1418, anti-PRO1474, anti-PRO1917, anti-PRO5723, anti-PRO4405, anti-PRO4302, anti-PRO9940 or anti-PRO6006 antibody.

25. A method of diagnosing an immune-related disease in a mammal which comprises detecting  
20 the presence or absence of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide in a test sample of tissue cells obtained from said mammal, wherein the presence or absence of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333,  
25 PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide in said test sample is indicative of the presence of an immune-related disease in said mammal.

30 26. A method for identifying an agonist of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide comprising:

(a) contacting cells and a test compound to be screened under conditions suitable for the induction of a  
35 cellular response normally induced by a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective  
40 agonist, wherein the induction of said cellular response is indicative of said test compound being an effective



agonist.

27. A method for identifying an agonist of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide comprising:

(a) contacting cells and a test compound to be screened under conditions suitable for the stimulation of cell proliferation by a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide; and

(b) measuring the proliferation of said cells to determine if the test compound is an effective agonist, wherein the stimulation of cell proliferation is indicative of said test compound being an effective agonist.

28. A method for identifying a compound capable of inhibiting the expression and/or activity of a PRO polypeptide by contacting a candidate compound with a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide under conditions and for a time sufficient to allow these two components to interact and determining whether the activity of the PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide is inhibited.

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29. The method of claim 28 comprising the steps of:

(a) contacting cells and a test compound to be screened in the presence of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide under conditions suitable for the induction of a cellular response normally induced by a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide; and

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(b) determining the induction of said cellular response to determine if the test compound is an effective antagonist.

30. The method of claim 28 comprising the steps of:

(a) contacting cells and a test compound to be screened in the presence of a PRO184, PRO212,

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PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide under conditions suitable for the stimulation of cell proliferation by a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide under conditions suitable for the stimulation of cell proliferation by a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide; and

(b) measuring the proliferation of the cells to determine if the test compound is an effective antagonist.

31. A method for identifying a compound that inhibits the expression of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide in cells that normally express the polypeptide, wherein the method comprises contacting the cells with a test compound and determining whether the expression of the PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide is inhibited.

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32. The method of claim 31 comprising the steps of:

(a) contacting cells and a test compound to be screened under conditions suitable for allowing expression of the PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide; and

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(b) determining the inhibition of expression of said polypeptide.

33. A method for treating an immune-related disorder in a mammal that suffers therefrom comprising administering to the mammal a nucleic acid molecule that codes for either (a) a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, (b) and agonist of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031,

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PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide or (c) an antagonist of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide.

34. The method of claim 33 wherein said agonist or antagonist is an anti-PRO184, anti-PRO212, anti-PRO245, anti-PRO266, anti-PRO306, anti-PRO333, anti-PRO526, anti-PRO381, anti-PRO364, anti-PRO356, anti-PRO719, anti-PRO861, anti-PRO769, anti-PRO788, anti-PRO826, anti-PRO982, anti-PRO779, anti-PRO1068, anti-PRO1031, anti-PRO1157, anti-PRO1159, anti-PRO1475, anti-PRO1271, anti-PRO1343, anti-PRO1375, anti-PRO1418, anti-PRO1474, anti-PRO1917, anti-PRO5723, anti-PRO4405, anti-PRO4302, anti-PRO9940 or anti-PRO6006 antibody.

35. The method of claim 33 the nucleic acid is administered via *ex vivo* gene therapy.

36. The method of claim 35 wherein the nucleic acid is comprised within a vector.

37. The method of claim 36 wherein the vector is selected from the group consisting of: an adenoviral, a adeno-associated viral, a lentiviral and a retroviral vector.

38. A retroviral vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, (b) an agonist polypeptide of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein the retroviral vector is in association with retroviral structural proteins.

39. An *ex vivo* producer cell comprising a nucleic acid construct that expresses retroviral structural proteins and also comprises a retroviral vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide, (b) an agonist polypeptide or a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343,

PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein said producer cell packages the retroviral vector in association with the structural proteins to produce recombinant retroviral particles.

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40. A method of stimulating the proliferation of T-lymphocytes in a mammal comprising administering a therapeutically effective amount of a PRO861, PRO788, PRO1159, PRO1646, PRO1475, PRO1917, PRO9940, PRO5723 or PRO6006 polypeptide, wherein the proliferation of T-lymphocytes in the mammal is stimulated.

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41. A method of decreasing the proliferation of T-lymphocytes in a mammal comprising administering a therapeutically effective amount of a PRO184, PRO306, PRO779, PRO1271, PRO1375 or PRO1474 polypeptide, wherein the proliferation of T-lymphocytes in the mammal is decreased.

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42. A method of stimulating the activity of T-lymphocytes comprising administering a therapeutically effective amount of a PRO245, PRO266, PRO306, PRO333, PRO356, PRO364, PRO381, PRO526, PRO719, PRO769, PRO826, PRO1031, PRO1069, PRO1343, PRO1375 or PRO1418 polypeptide, wherein the activity of T-lymphocytes is increased.

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43. A method of decreasing the activity of T-lymphocytes comprising administering a therapeutically effective amount of a PRO184, PRO212, PRO306, PRO333, PRO364, PRO381, PRO982, PRO1068, PRO1157, wherein the activity of T-lymphocytes is decreased.

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44. A method of affecting the proliferation of T-cells comprising contacting PBMC cells with an effective amount of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940, PRO6006 polypeptide and measuring the change in proliferation from control levels.

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45. A method of stimulating the activity of T-cells comprising contacting CD4+ cells or PBMC cells with an effective amount of a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940, PRO6006 polypeptide in combination with an effective amount of anti-CD3 antibody and measuring the change in activity from control levels.

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46. A method of inhibiting the activity of T-cells comprising contacting CD4+ cells which have been previously stimulated by treatment with anti-CD3 and anti-CD28 antibodies, with an effective amount of PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159,

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PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940, PRO6006 polypeptide and measuring the change in activity from control levels.

47. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:3), Figure 5 (SEQ ID NO:8), Figure 7 (SEQ ID NO:13), Figure 9 (SEQ ID NO:18), Figure 11 (SEQ ID NO:26), Figure 13 (SEQ ID NO:28), Figure 15 (SEQ ID NO:33), Figure 17 (SEQ ID NO:38), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:53), Figure 23 (SEQ ID NO:58), Figure 25 (SEQ ID NO:60), Figure 27 (SEQ ID NO:66), Figure 29 (SEQ ID NO:68), Figure 31 (SEQ ID NO:70), Figure 33 (SEQ ID NO:72), Figure 35 (SEQ ID NO:76), Figure 37 (SEQ ID NO:78), Figure 39 (SEQ ID NO:80), Figure 41 (SEQ ID NO:82), Figure 43 (SEQ ID NO:84), Figure 45 (SEQ ID NO:91), Figure 47 (SEQ ID NO:93), Figure 49 (SEQ ID NO:98), Figure 51 (SEQ ID NO:100), Figure 53 (SEQ ID NO:102), Figure 55 (SEQ ID NO:104), Figure 57 (SEQ ID NO:106), Figure 59 (SEQ ID NO:108), Figure 61 (SEQ ID NO:113), Figure 63 (SEQ ID NO:115) or Figure 65 (SEQ ID NO:117).

48. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:3), Figure 5 (SEQ ID NO:8), Figure 7 (SEQ ID NO:13), Figure 9 (SEQ ID NO:18), Figure 11 (SEQ ID NO:26), Figure 13 (SEQ ID NO:28), Figure 15 (SEQ ID NO:33), Figure 17 (SEQ ID NO:38), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:53), Figure 23 (SEQ ID NO:58), Figure 25 (SEQ ID NO:60), Figure 27 (SEQ ID NO:66), Figure 29 (SEQ ID NO:68), Figure 31 (SEQ ID NO:70), Figure 33 (SEQ ID NO:72), Figure 35 (SEQ ID NO:76), Figure 37 (SEQ ID NO:78), Figure 39 (SEQ ID NO:80), Figure 41 (SEQ ID NO:82), Figure 43 (SEQ ID NO:84), Figure 45 (SEQ ID NO:91), Figure 47 (SEQ ID NO:93), Figure 49 (SEQ ID NO:98), Figure 51 (SEQ ID NO:100), Figure 53 (SEQ ID NO:102), Figure 55 (SEQ ID NO:104), Figure 57 (SEQ ID NO:106), Figure 59 (SEQ ID NO:108), Figure 61 (SEQ ID NO:113), Figure 63 (SEQ ID NO:115) or Figure 65 (SEQ ID NO:117).

49. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the full-length coding sequence of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:3), Figure 5 (SEQ ID NO:8), Figure 7 (SEQ ID NO:13), Figure 9 (SEQ ID NO:18), Figure 11 (SEQ ID NO:26), Figure 13 (SEQ ID NO:28), Figure 15 (SEQ ID NO:33), Figure 17 (SEQ ID NO:38), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:53), Figure 23 (SEQ ID NO:58), Figure 25 (SEQ ID NO:60), Figure 27 (SEQ ID NO:66), Figure 29 (SEQ ID NO:68), Figure 31 (SEQ ID NO:70), Figure 33 (SEQ ID NO:72), Figure 35 (SEQ ID NO:76), Figure 37 (SEQ ID NO:78), Figure 39 (SEQ ID NO:80), Figure 41 (SEQ ID NO:82), Figure 43 (SEQ ID NO:84), Figure 45 (SEQ ID NO:91), Figure 47 (SEQ ID NO:93), Figure 49 (SEQ ID NO:98), Figure 51 (SEQ ID NO:100), Figure 53 (SEQ ID NO:102), Figure 55 (SEQ ID NO:104), Figure 57 (SEQ ID NO:106), Figure 59 (SEQ ID NO:108), Figure 61 (SEQ ID NO:113), Figure 63 (SEQ ID NO:115) or Figure 65 (SEQ ID NO:117).

50. Isolated nucleic acid having at least 80% nucleic acid sequence identity to the full-length

5 51. A vector comprising the nucleic acid of any one of Claims 47 to 50.

52. The vector of Claim 51 operably linked to control sequences recognized by a host cell transformed with the vector.

10 53. A host cell comprising the vector of Claim 51.

54. The host cell of Claim 53 wherein said cell is a CHO cell.

55. The host cell of Claim 53, wherein said cell is an *E. coli*.

15 56. The host cell of Claim 53, wherein said cell is a yeast cell.

57. A process for producing a PRO184, PRO212, PRO245, PRO266, PRO306, PRO333, PRO526, PRO381, PRO364, PRO356, PRO719, PRO861, PRO769, PRO788, PRO826, PRO982, PRO779, PRO1068, PRO1031, PRO1157, PRO1159, PRO1475, PRO1271, PRO1343, PRO1375, PRO1418, PRO1474, PRO1917, PRO5723, PRO4405, PRO4302, PRO9940 or PRO6006 polypeptide comprising culturing the host cell of Claim 53 under conditions suitable for expression of said polypeptide and recovering said polypeptide from the cell culture.

20 58. An isolated polypeptide having at least 80% amino acid sequence identity to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118).

25 59. An isolated polypeptide scoring at least 80% positives when compared to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118).

30 60. An isolated polypeptide scoring at least 80% positives when compared to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118).

35 61. An isolated polypeptide scoring at least 80% positives when compared to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118).

NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94),  
 5 Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118).

60. An isolated polypeptide having at least 80% amino acid sequence identity to an amino acid  
 10 sequence encoded by the full-length coding sequence of the DNA deposited under ATCC accession number 209254, 209265, 209401, 209435, -----, 209704, 209808, 209436, 209422, 209705, -----, 209849, 203017, 203583, 55820, 203046, 209866, 203540, 203092, 203464, 203235, 203282, 203115, 203276, 203270, 203573, 203966, 203834 or 387-PTA.

15 61. A chimeric molecule comprising a polypeptide according to any one of Claims 58 to 60 fused to a heterologous amino acid sequence.

20 62. The chimeric molecule of Claim 61, wherein said heterologous amino acid sequence is an epitope tag sequence.

63. The chimeric molecule of Claim 61, wherein said heterologous amino acid sequence is a Fc region of an immunoglobulin.

25 64. An antibody which specifically binds to a polypeptide according to any one of Claims 58 to 60.

65. The antibody of Claim 64, wherein said antibody is a monoclonal antibody, a humanized antibody or a single-chain antibody.

30 66. Isolated nucleic acid having at least 80% nucleic acid sequence identity to:  
 (a) a nucleotide sequence encoding the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61),  
 35 Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID  
 40 NO:116) or Figure 66 (SEQ ID NO:118) lacking its associated signal peptide;

(b) a nucleotide sequence encoding an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118) with its associated signal peptide; or

(c) a nucleotide sequence encoding an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118) lacking its associated signal peptide.

67. An isolated polypeptide having at least 80% amino acid sequence identity to:

(a) the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118) lacking its associated signal peptide;

(b) an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58



(SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118), with its associated signal peptide; or

- (c) an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:19), Figure 12 (SEQ ID NO:27), Figure 14 (SEQ ID NO:29), Figure 16 (SEQ ID NO:34), Figure 18 (SEQ ID NO:39), Figure 20 (SEQ ID NO:49), Figure 22 (SEQ ID NO:54), Figure 24 (SEQ ID NO:59), Figure 26 (SEQ ID NO:61), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:69), Figure 32 (SEQ ID NO:71), Figure 34 (SEQ ID NO:73), Figure 36 (SEQ ID NO:77), Figure 38 (SEQ ID NO:79), Figure 40 (SEQ ID NO:81), Figure 42 (SEQ ID NO:83), Figure 44 (SEQ ID NO:85), Figure 46 (SEQ ID NO:92), Figure 48 (SEQ ID NO:94), Figure 50 (SEQ ID NO:99), Figure 52 (SEQ ID NO:101), Figure 54 (SEQ ID NO:103), Figure 56 (SEQ ID NO:105), Figure 58 (SEQ ID NO:107), Figure 60 (SEQ ID NO:109), Figure 62 (SEQ ID NO:114), Figure 64 (SEQ ID NO:116) or Figure 66 (SEQ ID NO:118), lacking its associated signal peptide.

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Figure 1

CCAGGATGGAGCTGGGGCCTGTATAGCCATATTATTGTTCTATGCTACTAGACATGGGGGGGACTTGGTGAAAA  
AGGTATTATCCAGCCAGAGGGTCTGGGAGCCCTGTCTTACTGAACCTGGGCAACCTGGATATTCTGAGACATAT  
TTTGGGGGGGATTTCAGTGAAAAAAGTGGGGGATCCCTCCATTTAGAGTGTAGCAAAGGAAAAAACACCAAGGT  
TGGGTTCTTCTGACATTGGCAGTGCCCCAGTAGGGGTGGGATGAGCGAATATTCCCAAAGCTAAAGTCCAC  
ACCCTGTAGATTACAAGAGTGGATTTGGCAGGAGTGTGCCCCAAAATACAGTGGAAGGTGCCTGAAGATATTT  
AAACCACGTCTTGGAATTTAGTGGGTCTTGGCTTTGGGATAGGTGAAGTGAGGACAGACACTGGAGAGGAGGG  
AAAGGGGACGTTTTCAATAGGAGGCAAACTCGAGGGTGGGATCCACTGAGGAGTACATAGGCTGCTGGATCTG  
GTGGAGCCAGCACTGGGCCCACGGGTGGTAAGTGGCTGCTGTGGAGGGGGGTACGTGAGGGGGGGTCTGGGGC  
TTATCCTCAGGTCCTGTGGGTGGGGCAGCGAGTCGGGGCCTGAGCGTCAAGAGCATGCCCTAGTGAGCGGGCTC  
CTCTGGGGGAGCCCAGCGCGCTCCGGGCGCCTGCCGTTTGGGGGTGTCTCCTCCCGGGGCGCTATGGCGGCGC  
TGGCCAGTAGCCTGATCCGGCAGAAGCGGGAGGTCCGCGAGCCCGGGGGCAGCCGGCCGGTGTCCGGCGCAGCGG  
CGCGTGTGTCCCGCGGCACCAAGTCCCTTTGCCAGAAGCAGCTCCTCATCTGCTGTCCAAGGTGCCACTGTG  
CGGGGGGCGGCCGCGCGGCCGACCGCGGCCCGGAGCCTCAGCTCAAAGGCATCGTCACCAAAGTGTCTGCC  
GCCAGGGTTTCTACCTCCAGGCGAATCCCGACGGAAGCATCCAGGGCACCCAGAGGATACCAGCTCCTTCACC  
CACTTCAACCTGATCCCTGTGGGCCTCCGTGTGGTCACCATCCAGAGCGCCAAGCTGGGTCACTACATGGCCAT  
GAATGCTGAGGGACTGCTCTACAGTTCGCGCATTTACAGCTGAGTGTGCTTTAAGGAGTGTGTCTTTGAGA  
ATTACTACGTCTGTACGCCTCTGCTCTCTACCGCCAGCGTCGTCTTGGCCGGGCCTGGTACCTCGGCCTGGAC  
AAGGAGGGCCAGGTCATGAAGGGAACCGAGTTAAGAAGACCAAGGCAGCTGCCCACTTTCTGCCCCAAGCTCCT  
GGAGTGGCCATGTACCAGGAGCCTTCTCTCCACAGTGTCCCCGAGGCCTCCCTTCCAGTCCCCCTGCCCCCT  
GAAATGTAGTCCCTGGACTGGAGGTTCCCTGCACTCCCACTGAGCCAGCCACCACCACACCTGT

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Figure 2

MAALASSLIHQKREVREPGGSRPVSAQRRVCPRGTKSLCQKQLLILLSKVRLCGGRPARPDGRGPEPQLKGIVTK  
LFCRQGFYLANPDGSIQGTPEDTSSFTFNLI PVGLRVVTIQSAXLGHYMAMNAEGLLYSSPHFTAECRFKEC  
VFENYYVLYASALYRQRSGRAWYLGLDKEGQVMKGNRVKKTAAAHLPLKLEVAMYQEPSLHSVPEASPPSP  
PAP

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Figure 3

TCCGCAGGCGCACC GGGGGCAAAGGAGGTGGCATGTGGTCAGGCACAGCAGGGTCCTGTGTCCGCGCTGAGCC  
GCGCTCTCCCTGCTCCAGCAAGGACCATGAGGGCGCTGGAGGGGCCAGGCCTGTGCTGCTGTGCCTGGTGTG  
GCGCTGCCTGCCCTGCTGCCGGTGCCGGCTGTACGCGGAGTGGCAGAAACACCCACCTACCCCTGGCGGGACGC  
AGAGACAGGGGAGCGGCTGGTGTGCGCCAGTGCCCCCAGGCACCTTTGTGCAGCGGCCGTGCCGCCGAGACA  
GCCCCACGACGTGTGGCCCGTGTCCACCGCGCCACTACACGCAGTTCTGGAACTACCTGGAGCGCTGCCGCTAC  
TGCAACGTCTCTGCCGGGAGCGTGAGGAGGAGGCACGGGCTTGCCACGCCACCCACAACCGTGCCTGCCGCTG  
CCGCACCGGCTTCTTCGCGCACGCTGGTTTCTGCTTGGAGCACGCATCGTGTCCACCTGGTGCCGGCGTGATTG  
CCCCGGGCACCCCCAGCCAGAACACGCAGTGCCAGCCGTGCCCCCAGGCACCTTCTCAGCCAGCAGCTCCAGC  
TCAGAGCAGTGCCAGCCCCACCGCAACTGCACGGCCCTGGGCCCTGGCCCTCAATGTGCCAGGCTCTTCCTCCCA  
TGACACCTGTGCACCAGCTGCACTGGCTTCCCCCTCAGCACCAGGGTACCAGGAGCTGAGGAGTGTGAGCGTG  
CCGTCAATCGACTTTGTGGCTTTCAGGACATCTCCATCAAGAGGCTGCAGCGCTGCTGCAGGCCCTCGAGGCC  
CCGGAGGGCTGGGGTCCGACACCAAGGGCGGGCCGCGGGCCTTGCACTGAAGCTGCGTCGGCGGCTCACGGA  
GCTCCTGGGGGCGCAGGACGGGGCGCTGCTGGTGGCGCTGCTGCAGGCGCTGCGCGTGGCCAGGATGCCCCGGC  
TGGAGCGGAGCGTCCGTGAGCGCTTCTCCCTGTGCACATCCTTGGCCCCCTCTTATTTATTCTACATCCTTG  
GCACCCCACTTGCACTGAAAGAGGCTTTTTTTTAAATAGAAGAAATGAGGTTCTTAAAAAAAAAAAAAAAAAA  
AAAA

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Figure 4

MRLEGPGLSLLCLVLALPALLPVPVAVRGVAETPTYFWRDAETGERLVCAQCPPGTFVQRPCCRDSPTTCGPCP  
PRHYTQFWNYLERCRYCNVLCGEREEEARACHATHNRACRCRTGFFAHAGFCLEHASCPPGAGVIAPGTPSQNT  
QCQPCPPGTFSASSSSSEQCQPHRNCTALGLALNVPGSSSHDTLCTSCTGFPLSTRVPGAEECERAVIDFVAFQ  
DISIKRLQRLQLALEAPEGWGPTPRAGRAALQLKLRRRLTELLGAQDGALLVRLQLALRVARMPGLERSVREF  
LPVH

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Figure 5

CCGAGAAGTTC AAGGGCCCCGCGCTCCTGCGCTCCTGCCGCCGGGACCCTCGACCTCCTCAGAGCAGCCGGCT  
GCGGCCCGGGAAGATGGCGAGGAGGAGCGGCCACCGCTCCTCCTGCTGCTGCTGCGCTACCTGGTGGTGGCC  
CTGGGCTATCATAAGGCCTATGGGTTTTCTGCCCCAAAGACCAACAAGTAGTCACAGCAGTAGAGTACCAAGA  
GGCTATTTTAGCCTGCAAACCCCAAAGAAGACTGTTCTCCAGATTAGCTGGAAGAACTGGGTCCGAGTG  
TCTCCTTTGTCTACTATCAACAGACTCTTCAAGGTGATTTTAAAAATCGAGCTGAGATGATAGATTTCATATC  
CGGATCAAAATGTGACAAGAACTGATCGGGGAAATATCGTTGTGAAGTTAGTGCCCCATCTGAGCAAGGCCA  
AAACCTGGAAGAGGATACAGTCACCTCTGGAAGTATTAGTGGTCCAGCAGTTCCATCATGTGAAGTACCCTCTT  
CTGCTCTGAGTGGAACCTGTGGTAGAGCTACGATGTCAAGACAAAGAAGGGAATCCAGCTCCTGAATACACATGG  
TTTAAGGATGGCATCCGTTTGTAGAAAATCCAGACTTGGCTCCCCAAGCACCACAGCTCATACAATGAA  
TACAAAACCTGGAACCTCTGCAATTTAATACTGTTTCCAACTGGACACTGGAGAATTTCTGTGAAGCCCGCA  
ATTCTGTGGATATCGCAGGTGCTCTGGAAACGAATGCAAGTAGATGATCTCAACATAAGTGGCATCATAGCA  
CCCGTAGTAGTTTGTGGCCTTAGTGATTTCCGTTTGTGGCCTTGTTGTATGCTATGCTCAGAGGAAAGGCTACTT  
TTCAAAGAAACCTCCTTCCAGAAGAGTAATTCTTCATCTAAAGCCACGACAATGAGTGAAAATGTGCAGTGGC  
TCAGCCTGTAATCCCAGCACCTTTGGAAGGCCGCGCGGGCGGATCACGAGGTCAGGAGTTCTAGACCAGCTG  
GCCAATATGGTGAAACCCCATCTCTACTAAATACAAAATTAGCTGGGCATGGTGGCATGTGCTTGCAGTTCC  
AGTGCTTTGGGAGACAGGAGAACTCACTTGAAACCCGGAGGCGAGGTTGCAGTGAGCTGAGATCACGCCACTGC  
AGTCCAGCCTGGGTAAACAGCAAGATTCCATCTCAAAAATAAAATAAAATAAAATAAAATACTGGTTTTTA  
CCTGTAGAATTCTTACAATAAATATAGCTTGATATTC

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Figure 6

MARRSRHRLLLLLLRYLVVALGYHKAYGFSAPKDQQVVTAVEYQEAILACKTPKKTVSSRLEWKKLGRSVSFVY  
YQQTLOGDFKNRAEMIDFNIRIKNVTRSDAGKYRCEVSAPSEQGQNLEEDTVTLEVLVAPAVPSCEVPSSALSG  
TVVELRCQDKEGNPAPEYTWFKDGIRLLENPRLGSTNSSTMTKTGTLOFNTVSKLDTGEYSCEARNSVG  
RRCPGKRMQVDDLNIISGIIAAVVVVALVISVCGLGVCYAQRKGYFSKETSQKSNSSSKATTMSENVQWLTPVI  
PALWKAAGGSRGQEF

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Figure 7

GGGGGTAGGGAGGAAGGAATCCACCCCCACCCCCCAAACCCCTTTCTTCTCCTTTCTGGCTTCGGACATTG  
GAGCACTAAATGAACCTGAATTGTGTCTGTGGCGAGCAGGATGGTCGCTGTTACTTTGTGATGAGATCGGGGAT  
GAATTGCTCGCTTTAAAAATGCTGCTTTGGATTCTGTTGCTGGAGACGTCTCTTTGTTTTGCCGCTGGAACGT  
TACAGGGGACGTTTGCAAAGAGAAGATCTGTTCTGCAATGAGATAGAAGGGGACCTACACGTAGACTGTGAAA  
AAAAGGGCTTCACAGTCTGCAGCGTTTCTACTGCCCGACTTCCCAGTTTTACCATTTATTTCTGCATGGCAAT  
TCCCTCACTCGACTTTTCCCTAATGAGTTCGCTAATTTTATAATGCGGTAGTTTGCACATGGAACAATGG  
CTTGCAATGAAATCGTTCCGGGGGCTTTTCTGGGGCTGCAGCTGGTGAAAAGGCTGCACATCAACAACAACAAGA  
TCAAGTCTTTTTCGAAAGCAGACTTTTCTGGGGCTGGACGATCTGGAATATCTCCAGGCTGATTTTAATTTATTA  
CGAGATATAGACCCGGGGGCTTCCAGGACTTGAACAAGCTGGAGGTGCTCATTTTAAATGACAATCTCATCAG  
CACCTACCTGCCAACGTGTTCCAGTATGTGCCCATCACCCACCTCGACCTCCGGGGTAACAGGCTGAAAACGC  
TGCCCTATGAGGAGGTCTTGGAGCAAATCCCTGGTATTGCGGAGATCCTGCTAGAGGATAACCCCTTGGGACTGC  
ACCTGTGATCTGCTCTCCCTGAAAGAATGGCTGGAAAACATTCCCAAGAATGCCCTGATCGGCCGAGTGGTCTG  
CGAAGCCCCCAGAGCTGCAGGGTAAAGACCTCAATGAAACCACCGAACAGGACTTGTGTCTTTGAAAAACC  
GAGTGGATTCTAGTCTCCCGCGCCCCCTGCCCAAGAAGAGACCTTTGCTCCTGGACCCCTGCCAATCCTTTC  
AAGACAAATGGGCAAGAGGATCATGCCACACCAGGGTCTGCTCCAACCGAGGTACAAAGATCCCAGGCAACTG  
GCAGATCAAAATCAGACCCACAGCAGCGATAGCGACGGGTAGTCCAGGAACAAACCCCTTAGCTAACAGTTTAC  
CCTGCCCTGGGGGCTGCAGCTGCGACCATCCCAGGGTGGGTTTAAAGATGAAGTGAACAACAGGAACGTG  
AGCAGCTTGGCTGATTTGAAGCCCAAGCTCTCTAACGTGCAGGAGCTTTTCTACGAGATAACAAGATCCACAG  
CATCCGAAAAATCGCACTTTTGTGGATTACAAGAACCTCATTCTGTTGGATCTGGGCAACAATAACATCGCTACTG  
TAGAGAACAACACTTTCAAGAACCTTTTGGACCTCAGGTGGCTATACATGGATAGCAATTACCTGGACAGCTG  
TCCCGGGAGAAATTCGCGGGGCTGCAAAACCTAGAGTACCTGAACGTGGAGTACAACGCTATCCAGCTCATCCT  
CCCGGGCACTTTCAATGCCATGCCCAAACCTGAGGATCCTCATTCTCAACAACAACCTGCTGAGGTCCCTGCCTG  
TGGACGTGTTCTGCTGGGGTCTCGCTCTCTAACTCAGCCTGCACAACAATTACTTCATGTACCTCCCGGTGGCA  
GGGGTGTCTGGACCAAGTTAACCTCCATCATCCAGATAGACCTCCACGGAACCCCTGGGAGTGCTCCTGCACAAT  
TGTGCCTTTCAAGCAGTGGGCAGAACGCTTGGGTTCGGAAGTGCTGATGAGCGACCTCAAGTGTGAGACGCCGG  
TGAATCTCTTAGAAAGGATTTTATGCTCCTCTCCAATGACGAGATCTGCCCTCAGCTGTACGCTAGGATCTCG  
CCCACGTTAACTTCGCACAGTAAAAACAGCACTGGGTGGCGGAGACCGGGACGCACTCCAACCTCCTACCTAGA  
CACCAGCAGGGTGTCCATCTCGGTGTTGGTCCCGGACTGCTGCTGGTGTGTTGTACCTCCGCTTCCACGCTGG  
TGGCATGCTCGTGTATCCTGAGGAACCGAAAGCGTCCAAGAGACGAGATGCCAACTCCTCCGCGTCCGAG  
ATTAATTCCTACAGACAGTCTGTGACTCTTCTACTGGCACAATGGGCCTTACAACGCAGATGGGGCCACAG  
AGTGTATGACTGTGGCTCTCACTCGCTCTCAGACTAAGACCCCAACCCCAATAGGGGAGGGCAGAGGGAAGGCG  
ATACATCCTTCCCCACCGCAGGCACCCCGGGGCTGGAGGGGCGTGTACCCAAATCCCCGCGCCATCAGCCTGG  
ATGGGCATAAGTAGATAAAATACTGTGAGCTCGCACAAACCGAAAGGGCCTGACCCCTTACTTAGCTCCCTCCTT  
GAAACAAAGAGCAGACTGTGGAGAGCTGGGAGAGCGCAGCCAGCTCGCTCTTTGCTGAGAGCCCCCTTTGACAG  
AAAGCCCAGCACGACCCTGCTGGAAGAACTGACAGTGCCTCGCCCTCGGCCCGGGGCTGTGGGGTGGATG  
CCGCGGTTCTATACATATATACATATATCCACATCTATATAGAGAGATAGATATCTATTTTCCCCTGTGGATT  
AGCCCCGTGATGGCTCCCTGTTGGCTACGCAGGGATGGGCAGTTGCACGAAGGCATGAATGTATTGTAATAAG  
TAACCTTGACTTCTGAC



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Figure 8

MLLWILLLETSLCFAAGNVTGDVCKEKICSCNEIEGDLHVDCEKKGFTSLQRFTAPT SQFYHLFLHGNSLTRLF  
PNEFANFYNAVSLHMENNGLHEIVPGAFLGLQLVKRLHINNKKIKSFRKQTFGLDDLEYLQADFNLLRDIDPG  
AFQDLNKLEVLILNDNLISTLPANVFQYVPITHLDLRGNRLKTLPYEEVLEQIPGIAEILLEDNPWDCTCDLLS  
LKEWLENIPKNALIGRVVCEAPTRLQGKDLNETTEQDLCPLKNRVDSSLPAPPAQEETFAPGPLPTPFKTNGQE  
DHATPGSAPNGGTKIPGNWQIKIRPTAAIATGSSRNKPLANSRPCPGGCSCDHIPGSGLKMNCCNNRVSSSLADL  
KPKLSNVQELFLRDNKIHSIRKSHFVDYKNLILLDLGNNNIATVENNTFYNNLLDLRWLYMDSNYLDTLSREKFA  
GLQNLEYLNVEYNAIQILIPGTFNAMPKLRILILNNNLLRSLPVDVFAGVSLSKLSLHMNYFMYLPVAGVLDQL  
TSIIQIDLHGNPWECSTIVPFKQWAERLGSEVLMSDLKCETPVNFFRKDFMLLSNDEICPOLYARISPTLTSH  
SKNSTGLAETGTHSNSYLDTSRVSISVLVPGLLLVFVTSFTVVGMLVFILNRNRKRSKRRDANSSASEINSLQT  
VCDSSYWHNGPYNADGAHRVYDCGSHSLSD

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Figure 9

GTGCTGGGCTTTTTTCAGACAAGTGCATCTCCTAACCAGGTCACATTTTCAGCCGCGACCCACTCTCCGCCAGTCA  
CCGGAGGCAGACCGCGGGAGGAGAGCTGAGGACAGCCGCTGCGCTTCGCCAGCAGCGGGGTGGGAGGAAGGAC  
ATTAAAATACTGCAGAAAGTCAAGACCCCCCAGGTGCAACCCAGACCACGATGCGCGCCCCGGGCTGCGGGCGG  
CTGGTGCTGCCGCTGCTGCTCCTGGCCCGCGGCAGCCCTGGCCGAAGGCGACGCCAAGGGGCTCAAGGAGGGCGA  
GACCCCCGGCAATTTTCATGGAGGACGAGCAATGGCTGTCTCATCTCGCAGTACAGCGGCAAGATCAAGCACT  
GGAACCGCTTCCGAGACGAAGTGGAGGATGACTATATCAAGAGCTGGGAGGACAATCAGCAAGGAGATGAAGCC  
CTGGATACCACCAAGGACCCCTGCCAGAAGGTGAAGTGCAGCCGCCACAAGGTGTGCATTGCCCAGGGCTACCA  
GCGGGCCATGTGCATCAGTCGCAAGAAGCTGGAGCACAGGATCAAGCAGCCGACCGTGAAACTCCATGGAAACA  
AAGACTCCATCTGCAAGCCCTGCCACATGGCCAGCTTGCCCTGTCTGCGGGCTCAGATGGCCACACTTACAGC  
TCTGTGTGTAAGCTGGAGCAACAGGCGTGCCTGAGCAGCAAGCAGCTGGCGGTGCGATGCGAGGGCCCTGCC  
CTGCCCCACGGAGCAGGCTGCCACCTCCACCGCCGATGGCAAACCAGAGACTTGACCCGGTCAGGACCTGGCTG  
ACCTGGGAGATCGGCTGCGGGACTGGTTCCAGCTCCTTCATGAGAACTCCAAGCAGAATGGCTCAGCCAGCAGT  
GTAGCCGGCCCGGCCAGCGGGCTGGACAAGAGCCTGGGGGCCAGCTGCAAGGACTCCATTGGCTGGATGTTCTC  
CAAGCTGGACACCAGTGTGACCTCTTCTGGACCAGACGGAGCTGGCCGCCATCAACCTGGACAAGTACGAGG  
TCTGCATCCGTCCCTTCTTCAACTCCTGTGACACCTACAAGGATGGCCGGGTCTCTACTGCTGAGTGGTGCTTC  
TGCTTCTGGAGGGAGAAGCCCCCTGCCAGAGCTGGAGCGCATCCAGATCCAGGAGGCCGCCAAGAAGAA  
GCCAGGCATCTTCATCCCAGCTGCGACGAGGATGGCTACTACCGGAAGATGCAGTGTGACCAGAGCAGCGGTG  
ACTGCTGGCGTGTGGACCAGCTGGGCCTGGAGCTGACTGGCACGCGCACGCATGGGAGCCCCGACTGCCATGAC  
ATCGTGGGCTTCTCGGGGGACTTTGGAAGCGGTGTGCGCTGGGAGGATGAGGAGGAGAAGGAGACGGAGGAAGC  
AGGCGAGGAGGCCGAGGAGGAGGAGGGCGAGGCAGGCGAGGCTGACGACGGGGGCTACATCTGGTAGACGCCCT  
CAGGAGCCGGCTGCCGGGGGACTCAACAGCAGAGCTCTGAGCAGCAGCAGGCAACTTCGAGAACGGATCCAG  
AAATGCAGTCAGAAGGACCTGCTCCACCTGGGGGGACTGGGAGTGTGAGTGTGCATGGCATGTGTGTGGCACA  
GATGGCTGGGACGGGTGACAGTGTGAGTGCATGTGTGCATGCATGTGTGTATGTGTGTGTGTGTGGCATGCG  
CTGACAAATGTGTCCTTGATCCACACTGCTCCTGGCAGAGTGAGTCACCCAAAGGCCCTTCGGCCTCCTTGTA  
GCTGTTTTCTTTCTTTTGTGTTGTTTTAAATACATTACACACAAATACAAAAAAAAAAAAAAAAAAAAA  
AAA

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Figure 10

MRAPGCGRLVLPLLLLAAAAAEGDAKGLKEGETPGNFMEDQWLSSI SQYSGKIKHWNFRDEVEDDYIKSWE  
DNQQGDEALDTTKDPCQKVKCSRHKVCIAQGYQAMCISRKKLEHRIKQPTVKLHGKDSICKPCHMAQLASVC  
GSDGHTYSSVCKLEQQACLSSKQLAVRCEGPCPCPTEQAATSTADGKPETCTGQDLADLGDRLRDWFQLHENS  
KQNGSASSVAGPASGLDKSLGASCKDSIGWMFSKLDTSADLFLOQTELAAINLDKYEVCIRPFNSCDTYKDGR  
VSTAEWCFWREKPPCLAELEIRIQIEAAKKKPGIFIPSCDEDDGYRKMQCDQSSGDCWRVDQLGLELTGTRT  
HGSPDCDDIVGFSGDFGSGVGEDEEEKETEEAGEEAEDEEAGEAGEADDGGYIW

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Figure 11

TGCCCCCTGCTGCTGCTGCCCCCTGCTGTGGGGGGGGTCCCTGCAGGAGAAGCCAGTGTACGAGCTGCAAGTGCAG  
AAGTCGGTGACGGTGCAGGAGGGCCTGTGCGTCCCTTGTCCTTCTCTTACCCCTGGAGATCCTGGTA  
TTCCTCTCCCCACTCTACGTCTACTGGTTCCGGGACGGGAGATCCCATACTACGCTGAGGTTGTGGCCACAA  
ACAACCCAGACAGAAGAGTGAAGCCAGAGACCCAGGGCCGATTCCGCCTCCTTGGGGATGTCCAGAAGAAGAAC  
TGCTCCCTGAGCATCGGAGATGCCAGAATGGAGGACACGGGAAGCTATTTCTTCCGCGTGGAGAGAGGAAGGGA  
TGTAATAATAGCTACCAACAGAATAAGCTGAACTTGAGGTTGACAGCCCTGATAGAGAAACCCGACATCCACT  
TTCTGGAGCCTCTGGAGTCCGGCCGCCCAACAAGGCTGAGCTGCAGCCTTCCAGGATCCTGTGAAGCGGGACCA  
CCTCTCACATTCTCCTGGACGGGGAATGCCCTCAGCCCCCTGGACCCTGAGACCACCCGCTCCTCGGAGCTCAC  
CCTCACCCCCAGGCCCGAGGACCATGGCACCAACCTCACCTGTGAGGTGAAACGCCAAGGAGCTCAGGTGACCA  
CGGAGAGAACTGTCCAGCTCAATGTCTCCTATGCTCCACAGAACCTCGCCATCAGCATCTTCTTCAGAAATGGC  
ACAGGCACAGCCCTGCGGATCCTGAGCAATGGCATGTGGTGCCCATCCAGGAGGGCCAGTCCCTGTTCTCTCGC  
CTGCACAGTTGACAGCAACCCCCCTGCCTCACTGAGCTGGTTCCGGGAGGGGAAAAGCCCTCAATCCTTCCCAGA  
CCTCAATGTCTGGGACCCCTGGAGCTGCCTAACATAGGAGCTAGAGAGGGAGGGGAATTACCTGCCGGGTTTCAG  
CATCCGCTGGGCTCCCAGCACCTGTCTTCATCCTTTCTGTGCAGAGAAGCTCCTCTTCTCATATGTGTAAC  
TGAGAAACAGCAGGGTTCTTGCCCCCTCGTCTCACCTGATCAGGGGGGCTCTCATGGGGGCTGGCTTCTCTC  
TCACCTATGGCCTCACCTGGATCTACTATACCAGGTGTGGAGGCCCCAGCAGAGCAGGGCTGAGAGGCTGGC  
TGAGCCCCCTCCCGCTCAAGACAGAATTGAGGTGTGGACACTTAGCCCTGTGGGACACATGCAGGACATCACTGT  
CAGCTTCTTTCTGGAAGCTCACATCCCACTGACTACCCCTCTTTTCTTCTTCTGCCCCATACCCCTTCTACTTAT  
TCCCCTCTGCTTGTGAGTCTTGCCCCANACACCTGCATCCCCATCTGCANCCCATCCCCCTCTCCANCTGCCCT  
TCTCTTCCCTCTCCATCCANCATCTCCAGCCCTGTGAAGGGAATGTACTTTCCGGTCTATACCCCATTACCATT  
ACCAAAAGTTACCTTTTTTTTTTTTTT

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Figure 12

PLLLPLLLWGGSLQEKPVYELQVQKSVTVQEGLCVLVPCSFSPWRSWYSSPPLYVYWFRDGEIPYYAEVVA  
NPDRRVKQETQGRFRLLGDVQKKNCSLSIGDARMEDTGSYFFRVERGRDVKYSYQQNKLNEVTALIEKPD  
LEPLESGRPTRLSCSLPGSCEAGPPLTFSWTGNALSPLDPETTRSSSELTLPREPEDHGTNLTCQVKRGAQV  
ERTVQLNVSYAPQNLAISIFFRNGTGTALRILSNGMSVPIQEGQSLFLACTVDSNPPASLSWFEREGKALNPS  
MSGTLELPNIGAREGGEFTCRVQHPLGSQLSFILSVQRSSSSCICVTEKQGSWPLVLTIRGALMGAGFLL  
TYGLTWIYYTRCGGPQQSRAERPG

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Figure 13

GGCGCCGGTGCACCGGGCGGGCTGAGCGCCTCCTGCGGCCCGGCCCTGCGGCCCGGGCCCGCGCGCCGCCAC  
GCCCCAACCCCGGCCCGGCCCTAGCCCCCGCCGGGCCCGGCCCGGCCCGGCCCGGCCAGGTGAGCGCTCCG  
CCCCCGCGAGGCCCGGCCCGGCCCGGCCCGGCCCGGCCCGGCCCGGCCGAGCCGAGCCAGCCGCGCGG  
CGTCAAACCACTGATCCGATAAAACATTATCCTCCCGCGGCCCGCGCTGCGAGCGCCCGCCAGTCCGCGC  
CGCCCGCGCCCTCGCCCTGTGCGCCCTGCGCGCCCTGCGCACCCCGCGGCCCGAGCCAGCCAGCCGCGGGA  
GCGGAGCGCGCCGAGCCTCGTCCCGCGGCCCGGCCCGGCCCGGCCGCTAGCGCGCGCCCTGGATGCGGACCCG  
GCCCGCGGGGAGACGGGCGCCCGCCCGAAACGACTTTAGTCCCGACGCGCCCGCCCAACCCCTACGATGAA  
GAGGGCGTCCGCTGGAGGGAGCCGGCTGCTGGCATGGGTGCTGTGGCTGCAGGCCTGGCAGGTGGCAGCCCAT  
GCCCAGGTGCTGCGTATGCTACAATGAGCCCAAGGTGACGACAAGCTGCCCCAGCAGGGCCTGCAGGCTGTG  
CCCGTGGGCATCCCTGCTGCCAGCCAGCGCATCTTCTGACGCGCAACCGCATCTCGCATGTGCCAGTGCAG  
CTTCCGTGCTGCCGAACCTCACCATCTGTGGCTGCACTCGAATGTGCTGGCCCGAATTGATGCGGCTGCTT  
TCACTGGCCTGGCCCTCCTGGAGCAGCTGGACCTCAGCGATAATGCACAGCTCCGGTCTGTGGACCCCTGCCACA  
TTCCACGGCCTGGGCCGCTACACACGCTGCACCTGGACCGCTGCGGCCTGCAGGAGCTGGGCCCGGGGCTGTT  
CCGCGGCCTGGCTGCCCTGCAGTACCTCTACCTGCAGGACAACGCGCTGCAGGCACTGCCTGATGACACCTTCC  
GCGACCTGGGCAACCTCACACACCTCTTCTGACGCGCAACCGCATCTCCAGCGTGCCCGAGCGCGCCTTCCGT  
GGGCTGCACAGCCTCGACCGTCTCCTACTGCACACAGAACCGCTGGCCCATGTGCACCCGCATGCCTTCCGTGA  
CCTTGGCCGCTCATGACACTCTATCTGTTTGCCAACAATCTATCAGCGCTGCCCACTGAGGCCCTGGCCCCC  
TGCGTGCCCTGCAGTACCTGAGGCTCAACGACAACCCCTGGGTGTGTGACTGCCGGGCACGCCCCTCTGGGCC  
TGGCTGCAGAAGTTCCGCGGCTCCTCCTCCGAGGTGCCCTGCAGCCTCCCGCAACGCTGGCTGGCCGTGACCT  
CAAACGCTAGCTGCCAATGACCTGCAGGGCTGCGCTGTGGCCACCGGCCCTTACCATCCCATCTGGACCGGCA  
GGGCCACCGATGAGGAGCCGCTGGGGCTTCCCAAGTGTGCCAGCCAGATGCCGCTGACAAGGCCTCAGTACTG  
GAGCCTGGAAGACCAGCTTCGGCAGGCAATGCGCTGAAGGGACGCGTGCCGCCGCTGACAGCCCGCCGGGCAA  
CGGCTCTGGCCACGGCACATCAATGACTCACCTTTGGGACTCTGCCTGGCTCTGCTGAGCCCCGCTCACTG  
CAGTGGCGCCCGAGGGCTCCGAGCCACCAGGGTTCCCCACCTCGGGCCCTCGCCGGAGGGCCAGGCTGTTACGC  
AAGAACCGCACCCGAGCCACTGCCGTCTGGGCCAGGACGGCAGCGGGGTGGCGGGACTGGTGACTCAGAAGG  
CTCAGGTGCCCTACCCAGCCTCACCTGCAGCCTCACCCCTCGGGCTGGCGCTGGTGCTGTGGACAGTGCTTG  
GGCCCTGCTGACCCCCAGCGGACACAAGAGCGTGCTCAGCAGCCAGGTGTGTGTACATACGGGTCTCTCTCCA  
CGCCGCCAAGCCAGCCGGCGGCGGACCCGTGGGGCAGGCCAGGCCAGGTCTCCCTGATGGACGCTGCGGCC  
CGCCACCCCATCTCCACCCATCATGTTTACAGGGTTCGGCGGCAGCGTTTGTTCAGAACCGCGCTCCAC  
CCAGATCGCGGTATATAGAGATATGCATTTTATTTTACTTGTGTAAAAATATCGGACGACGTGGAATAAAGAGC  
TCTTTTCTTAAAAAA

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Figure 14

MKRASAGGSRLAWVLWLQAWQVAAPCPGACVCYNEPKVTTSCPQQGLQAVPVGIPAASQRIFLHGNRISHVPA  
ASFRACRNLTILWLHSNVLARIDAAFTGLALLEQLDLSDNAQLRSVDPATFHGLGRLHTLHLDRCGLELPGG  
LFRGLAALQYLYLQDNALQALPDDTFRDLGNLTHLFLHGNRISSVPERAFRGLHSLDRLLLHQNRAHVHVPFAF  
RDLGRLMTLYLFANNLSALPTEALAPLRALQYLRNDNPWVDCRARPLWAWLQKFRGSSSEVP CSLPQRLAGR  
DLKRLAANDLQGCATGPHYPIWTGRATDEEPLGLPKCCQPDAAADKASVLEPGRPASAGNALKGRVPPGDSPP  
GNGSGPRHINDSPFGTLPGSAEPPLTAVRPEGSEPPGFPTSGPRRRPGCSRKNRTRSHCRLGQAGSGGGGTGDS  
EGSGALPSLTCSLTPLGLALVLWTVLGPC

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Figure 15

TTCGTGACCCCTTGAGAAAAGAGTTGGTGGTAAATGTGCCACGTCTTCTAAGAAGGGGGAGTCCTGAACCTTGCTCT  
GAAGCCCTTGTCGGTAAGCCTTGAACCTACGTTCTTAAATCTATGAAGTCGAGGGACCTTTTCGCTGCTTTTGTAG  
GGACTTCTTTCTTCTGCTTCAGCAACATGAGGCTTTTCTTGTGGAACGCGGTCTTGACTCTGTTTCGTCACCTCTT  
TGATTGGGGCTTTGATCCCTGAACCAGAAGTGAAAATTGAAGTTCTCCAGAAGCCATTCTATCTGCCATCGCAAG  
ACCAAAGGAGGGGATTGATGTTGGTCCACTATGAAGGCTACTTAGAAAAGGACGGCTCCTTATTTCACTCCAC  
TCACAAACATAACAATGGTCAGCCCATTTGGTTTACCCTGGGCATCCTGGAGGCTCTCAAAGTTGGGACCAGG  
GCTTGAAGGAATGTGTGTAGGAGAGAAGAGAAAGCTCATCATTCCTCCTGCTCTGGGCTATGAAAAGAAGGA  
AAAGGTAAAATTCCCCAGAAAGTACACTGATATTTAATATTGATCTCCTGGAGATTGGAATGGACCAAGATC  
CCATGAATCATTCCAAGAAATGGATCTTAATGATGACTGGAACTCTCTAAAGATGAGGTTAAAGCATATTTAA  
AGAAGGAGTTTGA AAAACATGGTGGGTGGTGAATGAAAGTCATCATGATGCTTTGGTGGAGGATATTTTGTAT  
AAAGAAGATGAAGACAAAGATGGGTTTATATCTGCCAGAGAATTTACATATAAACACGATGAGTTATAGAGATA  
CATCTACCCCTTTTAAATATAGCACTCATCTTTCAAGAGAGGGCAGTCATCTTTAAAGAACATTTTATTTTATAC  
AATGTTCTTTCTTGCTTTGTTTTTATTTTTATATATTTTCTGACTCCTATTTTAAAGAACCCCTTAGGTTTC  
TAAGTACCCATTTCTTCTGATAAGTTATTGGGAAGAAAAGCTAATTGGTCTTTGAATAGAAGACTTCTGGAC  
AATTTTTCACTTTTACAGATATGAAGCTTTGTTTTACTTTCTCACTTATAAATTTAAAATGTTGCAACTGGGAA  
TATACCAGCATGAGACCAGGTTATAGCACAAATTAGCACCCCTATATTTCTGCTTCCCTCTATTTTCTCCAAG  
TTAGAGGTCAACATTTGAAAAGCCTTTTGCAATAGCCCAAGGCTTGCTATTTTCATGTTATAATGAAATAGTTT  
ATGTGTAACCTGGCTCTGAGTCTCTGCTTGAGGACCAGAGGAAAATGGTTGTTGGACCTGACTTGTAAATGGCTA  
CTGCTTTACTAAGGAGATGTGCAATGCTGAAGTTAGAAACAAGGTTAATAGCCAGGCATGGTGGCTCATGGCTG  
TAATCCCAGCACTTTGGGAGGCTGAGGCGGGCGCATCACCTGAGGTTGGGAGTTTCGAGACCAGCCTGACCAACA  
CGGAGAAACCTATCTCTACTAAAAATACAAAGTAGCCCGGCGTGGTGTGCGTGCCTGTAATCCCAGCTACCC  
AGGAAGGCTGAGGCGGCAGAAATCACTTGAACCCGAGGCCGAGGTTGCGGTAAGCCGAGATCACCTNCAGCCTGG  
ACACTCTGTCTCGAAAAAAGAAAAGAACACGGTTAATACCATATNAATATGTATGCATTGAGACATGCTACCTA  
GGACTTAAGCTGATGAAGCTTGGCTCCTAGTGATTTGGTGGCTATTATGATAAATAGGACAAATCATTTATGTG  
TGAGTTTCTTTGTAATAAAATGTATCAATATGTTATAGATGAGGTAGAAAGTTATATTTATATTCAATATTTAC  
TTCTTAAGGCTAGCGGAATATCCTTCTGTTCTTTAATGGGTAGTCTATAGTATATTATACTACAATAACATT  
GTATCATAAGATAAAGTAGTAAACAGTCTACATTTTCCATTTCTGTCTCATCAAAAACCTGAAGTTAGCTGGG  
TGTGGTGGCTCATGCCGTGAATCCCAGCACTTTGGGGGCCAAGGAGGTTGGATCACTTGAGATCAGGAGTTCAA  
GACCAGCCTGGCCAACATGGTGAACCTTGTCTCTACTAAAAATACAAAATTAGCCAGGCGGTGGTGGTGACA  
CCTGTAGTCCCAGCTACTCGGGAGGCTGAGACAGGAGATTGCTTGAACCCGGGAGGCGGAGGTTGCAGTGAGC  
CAAGATTGTGCCACTGCACTCCAGCCTGGGTGACAGAGCAAGACTCCATCTCAAAAAAAAAAAAAAGAAGCAGA  
CCTACAGCAGCTACTATTGAATAAAATACCTATCCTGGATTTT



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Figure 16

MRLFLWNAVLTLEFVTSLICALIPEPEVKIEVLQKPFICHRKTKGGDLMLVHYEGYLEKDGSLEHSTHKHNNQGP  
IWFTLGILEALKGWDQGLKGMCVGEKRLIIPPALGYGKEGKGKIPPESTLIFNIDLEIRNGPRSHESFQEMD  
LNDDWKLSKDEVKAYLKKEFEKHGAVVNESHHDALVEDIFDKEDEKDGFI SAREFTYKHDEL

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Figure 17

CACGCACTTCACCTGGGTTCGGGATTCCTCAGGTCATGAACGGTCCCAGCCACCTCCGGGCAGGGCGGGTGAGGAC  
GGGGACGGGGCGTGTCCAACCTGGCTGTGGGCTCTTGAAACCCGAGCATGGCACAGCACGGGGCGATGGGCGCGT  
TTCGGGCCCTGTGCGGCCTGGCGCTGCTGTGCGCGCTCAGCCTGGGTACGCGCCCCACCGGGGTCCCCGGTGC  
GGCCCTGGGCGCCTCCTGCTTGGGACGGGAACGGACGCGCGCTGCTGCCGGGTTCACACGACGCGCTGCTGCCG  
CGATTACCCGGGCGAGGAGTGCTGTTCCGAGTGGGACTGCATGTGTGTCCAGCCTGAATTCCTGCGGAGACC  
CTTGCTGCACGACCTGCCGGCACCACCCTTGTCCTCCAGGCCAGGGGGTACAGTCCCAGGGGAAATTCAGTTTT  
GGCTTCCAGTGTATCGACTGTGCCTCGGGGACETTCTCCGGGGGCCACGAAGGCCACTGCAAACCTTGGACAGA  
CTGCACCCAGTTCGGGTTTCTCACTGTGTTCCCTGGGAACAAGACCCACAACGCTGTGTGCGTCCCAGGGTCCC  
CGCCGGCAGAGCCGCTTGGGTGGCTGACCGTCGTCTCTGGCCGTGGCCGCTGCGTCTCTCCTGACCTCG  
GCCCAGCTTGGACTGCACATCTGGCAGCTGAGGAGTCAGTGCATGTGGCCCCGAGAGACCCAGCTGCTGCTGGA  
GGTGCCCGCTCGACCGAAGACGCCAGAAGCTGCCAGTTCCTCCGAGGAAGAGCGGGGCGAGCGATCGGCAGAGG  
AGAAGGGCGGCTGGGAGACCTGTGGGTGTGAGCCTGGCCGTCTCCGGGGCCACCGACCGCAGCCAGCCCTC  
CCCAGGAGCTCCCCAGGCCGACGGGGCTCTGCGTCTGCTCTGGGCCGGGCCCTGCTCCCCTGCCAGCAGAAGT  
GGGTGCAGGAAGGTGGCAGTGACCAGCGCCCTGGACCATGCAGTTC

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Figure 18

MAQHGA MGA FRALCGLALLCALS LGQRPTGGPGCGPGRLLLTGTGDARCCRVHTTRCCRDYPGEECCSEWDCMC  
VQPEFHCGDPCCTTCRHHPCPPGQGVQSQGKFSFGQCIDCASGTFSGGHEGHCKPWTDCIQFGFLTVEFGNKT  
HNAVCVPGSPPAEPLGWLTVVLLAVAACVLLLTSAQLGLHIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFPE  
EERGERSAEEKGRLGDLWV

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Figure 19

GAAAGCTATAGGCTACCCATTTCAGCTCCCCCTGTCAGAGACTCAAGCTTTGAGAAAGGCTAGCAAAGAGCAAGGA  
AAGAGAGAAAACAACAAAGTGGCGAGGCCCTCAGAGTGAAAGCGTAAGGTTTCAGTCAGCCTGCTGCAGCTTTGC  
AGACCTCAGCTGGGCATCTCCAGACTCCCCCTGAAGGAAGAGCCTTCCTCACCCPAACCCACAAAAGATGCTGAA  
AAAGCCTCTCTCAGCTGTGACCTGGCTCTGCATTTTCATCGTGGCCTTTGTTCAGCCACCCAGCGTGGCTGCAGA  
AGCTCTCTAAGCACAGACACCAGCACAGCCACAGCTCAAAGCGGCCAACTGCTGTGAGGAGGTGAAGGAGCTC  
AAGGCCCAAGTTGCCAACCTTAGCAGCCTGCTGAGTGAAGTGAACAAGAAGCAGGAGAGGGACTGGGTACGCGT  
GGTCATGCAGGTGATGGAGCTGGAGAGCAACAGCAAGCGCATGGAGTCGCGGCTCACAGATGCTGAGAGCAAGT  
ACTCCGAGATGAACAACCAAATTGACATCATGCAGCTGCAGGCAGCACAGACGGTCACTCAGACCTCCGCAGAT  
GCCATCTACGACTGCTCTTCCCTCTACCAGAAGAACTACCGCATCTCTGGAGTGTATAAGCTTCCTCCTGATGA  
CTTCTTGGGCAGCCCTGAACTGGAGGTGTTCTGTGACATGGAGACTTCAGGCGGAGGCTGGACCATCATCCAGA  
GACGAAAAAGTGGCCTTGTCTCCTTCTACCGGGACTGGGAAGCAGTACAAGCAGGGCTTTGGCAGCATCCGTGGG  
GACTTCTGGCTGGGGAACGAACACATCCACCGGCTCTCCAGACAGCCAACCCGGCTGCGTGTAGAGATGGAGGA  
CTGGGAGGGCAACCTGCGCTACGCTGAGTATAGCCACTTTGTTTTGGGCAATGAACTCAACAGCTATCGCCTCT  
TCCTGGGGAACCTACACTGGCAATGTGGGGAACACGCCCTCCAGTATCATAACAACACAGCCTTCAGCACCAAGG  
ACAAGGACAATGACAACCTGCTTGGACAAGTGTGCACAGCTCCGCAAGGTGGCTACTGGTACAACCTGCTGCACA  
GACTCCAACCTCAATGGAGTGTACTACCGCCTGGGTGAGCACAAATAAGCACCTGGATGGCATCACCTGGTATGG  
CTGGCATGGATCTACCTACTCCCTCAAACGGGTGGAGATGAAAAATCCGCCCAGAAGACTTCAAGCCTTAAAAGG  
AGGCTGCCCTGGAGCACGGATACAGAACTGAGACACGTGGAGACTGGATGAGGGCAGATGAGGACAGGAAGAG  
AGTGTTAGAAAGGGTAGGACTGAGAAACAGCCTATAATCTCCAAAGAAAGAATAAGTCTCCAAGGAGCACAAAA  
AAATCATATGTACCAAGGATGTTACAGTAAACAGGATGAAGTATTTAAACCCACTGGGTCTGCCACATCCTTC  
TCAAGGTGGTAGACTGAGTGGGTCTCTCTGCCCCAAGATCCCTGACATAGCAGTAGCTTGTCTTTCCACATGA  
TTTGTCTGTGAAAGAAAATAATTTGAGATCGTTTTATCTATTTTCTCTACGGCTTAGGCTATGTGAGGGCAAA  
ACACAAATCCCTTTGCTAAAAAGAACCATATTATTTTGATTCTCAAAGGATAGGCCCTTGAGTGTAGAGAAAG  
GAGTGAAGGAGGCAGGTGGGAAATGGTATTTCTATTTTTAAATCCAGTGAAATTATCTTGAGTCTACACATTAT  
TTTTAAACACAAAAATTGTTGCGCTGGAAGTGAACCCAGGCTGGACTTCGCGGGAGGAAATCCAGGGCACTGC  
ATCTGGCGATCAGACTCTGAGCACTGCCCCTCTGCTCGCCTTGGTCATGTACAGCACTGAAAGGAATGAAGCACA  
GCAGGAGGTGGACAGAGTCTCTCATGGATGCCGGCACAAAAGTGCCTTAAAAATATTCATAGTTAATACAGGTAT  
ATCTATTTTTATTTACTTTGTAAGAAACAAGCTCAAGGAGCTTCCTTTTAAATTTTGTCTGTAGGAAATGGTTG  
AAAAGTGAAGGTAGATGGTGTATAGTTAATAATAAATGCTGTAAATAAGCATCTCACTTTGTAAAAATAAAAT  
ATTGTGGTTTTGTTTTAAACATTCAACGTTTCTTTTCTCTACAATAAACACTTCAAAATGTG

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Figure 20

MLKKPLSAVTWLCIFIVAFVSHPAWLQKLSKHKTPAQPOLKAANCCEEVKELKAQVANLSSLLSELNKKQERDW  
VSVVMQVMELESNSKRME SRLTDAESKYSEMNNQIDIMQLQAAQTVTQTSADAIYDCSSLYQKNYRISGVYKLP  
PDDFLGSPELEVFCDMETSGGGWTIIQRRKSGLVSFYRDWKYKQGFGSIRGDFWLGNEHIHRLSRQPTRLRVE  
MEDWEGNLRVYAEYSHFVLCNELNSYRLFLGNYTGNVGN DALQYHNNTAFSTKDKDNDNCLDKCAQLRKGGYWYN  
CCTDSNLNGVYYRLGEHNKHLDGITWYGWHGSTYSLKRVEMKIRPEDFKP

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Figure 21

[illegible]

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Figure 22

MSNSVPLLCFWSLCYCFAAGSPVPFGPEGRLDKLHKPKATQTEVKPSVRFNLRRTSKDPEHEGCYLSVGHSQPL  
EDCSFNMTAKTFFLIHGTMSGIFENWLHKLVSALHTREKDANVVVDWLPLAHQLYTDVNNTRVVGHSIARM  
LDWLQEKDDFSLGNVHLIGYSLGAHVAGYAGNFVKGTVGRTGLDPAGPMFEGADHKRLSPDDADFVDVLHTY  
TRSFGLSIGIQMPVGHIDIYPNGGDFQPGCGLNDVLGSIAYGTITEVVKCEHERAVHLFVDSLQNDKPSFAFQ  
CTDSNRFKKGICLSCKNRCSIGYNAXKMRNKRNSKMYLKTRAGMPFRGNLQSLCP

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Figure 23

CGCCGCCCGCCGCTGCCTGGGCCGGGCCGAGGATGCGGCGCAGCGCCTCGGCGGCCAGGCTCGCTCCCCCTCCG  
GCACGCTGCTAACTTCCCCCGCTACGTCCCCGTTGCCCCGCCGGGCCGCCCGCTCTCCCCGCGCCCTCCGGGT  
CGGGTCTTCCAGGAGCGCCAGGCGCTGCCGCCGTGTGCCCTCCGCCGCTCGCCCGCGCGCCCGCGCTCCCCGCC  
TGCGCCCAAGCGCCCCGCGCCCGCGCCAGTCTCGGGCGGTCACTGCTGCCCTCTGCCTCGTGGCCGCCCTGTCT  
GCTGGCCGCCGGGCCCGGGCCGAGCCTGGGCGACGAAGCCATCCACTGCCCGCCCTGCTCCGAGGAGAAGCTGG  
CGCGCTGCCGCCCGCCCGTGGGCTGCGAGGAGCTGGTGCGAGAGCCGGGCTGCGGCTGTGCGCCACTTGGCGCC  
CTGGGCTTGGGGATGCCCTGCGGGGTGTACACCCCCGTTGCGGCTCGGGCCTGCGCTGCTACCCGCCCGGAGG  
GGTGGAGAAGCCCCCTGCACACACTGATGCACGGGAAGGCGTGTGCATGGAGCTGGCGGAGATCGAGGCCATCC  
AGGAAAGCCTGCAGCCCTCTGACAAGGACGAGGGTGACCACCCCAACAACAGCTTCAGCCCTGTAGCGCCCAT  
GACCGCAGGTGCTTGCAGAAGCACTTCGCCAAAATTGAGACCGGAGCACCAGTGGGGGCAAGATGAAGGTCAA  
TGGGGCGCCCCGGGAGGATGCCCGGCTGTGCCCCAGGGCTCCTGCCAGAGCGAGCTGCACCGGGCGCTGGAGC  
GGCTGGCCGCTTACAGAGCCGCACCCACGAGGACCTCTACATCATCCCCATCCCCAACTGCGACCGCAACGGC  
AACTTCCACCCCAAGCAGTGTACCCAGCTCTGGATGGGCAGCGTGGCAAGTGTGGTGTGGACCGGAAGAC  
GGGGGTGAAGCTTCCGGGGGGCCTGGAGCCAAAGGGGGAGCTGGACTGCCACCAGCTGGCTGACAGCTTTCGAG  
AGTGGAGCCTGCCAGCAGGCCAGGGACTCAGCGTCCCCCTGCTACTCCTGTGCTCTGGAGGCTGCAGAGCTGACC  
CAGAGTGGAGTCTGAGTCTGAGTCTGTCTCTGCTGCGGCCCAGAAGTTCCCTCAAATGCGCGTGTGCACGT  
GTGCGTGTGCGTGGT  
TCTTTGGTGTACACAGCCCAAGAGGACTGAGACTGGCACTTAGCCCAAGAGGTCTGAGCCCTGGTGTGTTC  
AGATCGATCTGGATTCACTCACTCACTCATTCTTCACTCATCCAGCCACCTAAAAACATTTACTGACCATGT  
ACTACGTGCCAGCTCTAGTTTTAGCCTTGGGAGGTTTTATTCTGACTTCTCTGATTTTGGCATGTGGAGACA  
CTCCTATAAGGAGAGTTCAAGCCTGTGGGAGTAGAAAAATCTCATTCCCAGAGTCAGAGGAGAAGAGACATGTA  
CCTTGACCATCGTCCTTCTCTCAAGCTAGCCAGAGGGTGGGAGCCTAAGGAAGCGTGGGGTAGCAGATGGAGT  
AATGGTCACGAGGTCCAGACCCACTCCCAAAGCTCAGACTTGCCAGGCTCCCTTTCTCTTCTTCCCCAGGTCT  
TCCTTTAGGTCTGGTTGTGACCATCTGCTTGGTTGGCTGGCAGCTGAGAGCCCTGCTGTGGGAGAGCGAAGG  
GGGTCAAAGGAAGACTTGAAGCACAGAGGGCTAGGGAGGTGGGTACATTTCTCTGAGCAGTCAGGGTGGGAAG  
AAAGAATGCAAGAGTGGACTGAATGTGCCTAATGGAGAAGACCCACGTGCTAGGGGATGAGGGGCTTCTGGGT  
CCTGTTCCCTACCCCATTTGTGGTCACAGCCATGAAGTCACCGGGATGAACCTATCCTTCCAGTGGCTCGCTCC  
CTGTAGCTCTGCCTCCCTCTCCATATCTCTTCCCTACACCTCCCTCCCCACACCTCCCTACTCCCTGGGCA  
TCTTCTGGCTTACTGGATGGAAGGAGACTTAGGAACCTACCAGTTGGCCATGATGTCTTTCTTCTTTTCTT  
TTTTTTAACAAAACAGAACAACCAAAAAATGTCCAAA



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Figure 24

MLPLCLVAALLLAAGPGPSLGDEAIHCPPCSEEXLARCRPPVVGCEELVREPGCGCCATCALGLGMPCGVYTPRC  
GSGLRICYPPRGVEKPLHTLMHGQGVCMELAEIEAIQESLQPSDKDEGDHPNNSFSPCSAHDRRCLOKHFAKIRD  
RSTSGGKMKVNGAPREDARPVFQGSCQSELHRALERLAASQSRTHEDLYIIPNCDRNGNFHPKQCHPALDGO  
RGKCWCVDRKTGVKLPGGLEPKGELDCHQLADSFRE

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Figure 25

GGCACGAGGGGGACAGGAGCTAATACCCAGAACTGAGTTGTGTCTGCTAAGTCCTCTGCCACGTACCCACGGG  
ATGAAGAACCTTTCATTTCCCTCCTTTTCCTTTTCTTCCTTGTCCTGAACTGCTGGGCTCCAGCATGCCACT  
GTGTCCCATCGATGAAGCCATCGACAAGAAGATCAAACAAGACTTCAACTCCCTGTTTCCAAATGCAATAAAGA  
ACATTGGCTTAAATTGCTGGACAGTCTCTCCAGAGGGAAGTTGGCCTCCTGCCCAGAAGGCACAGCAGTCTTG  
AGCTGCTCCTGTGGCTCTGCCTGTGGCTCGTGGGACATTCGTGAAGAAAAAGTGTGTCACTGCCAGTGTGCAAG  
GATAGACTGGACAGCAGCCCGCTGCTGTAAGCTGCAGGTCGCTTCCTGATGTCGGGGAAGTGAGCGTGGTTTCC  
AGCACAGCCACCCGTTCTGTAGCTCCAGAGATGTCTGATGTCTCCGGTCTCTACAGGCACCTGCACTCACGT  
GCGCGAATCCACACACAAGCACACATACTTAAAAATAAAACAAAACAGGCTGAAAAAAAAAAAAAAAAAAAAA  
AA

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Figure 26

MKNLSFPLLFLFFLVPELLGSSMPLCPIDEAIDKKIKQDFNSLFPNAIKNIGLNCWTVSSRGKLASCPEGTAVL  
SCSCGSACGSWDIREKVCHCQCARIDWTAARCCCKLQVAS

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Figure 27

CCAGTCTGTCGCCACCTCACTTGGTGTCTGCTGTCCCCGCCAGGCAAGCCTGGGGTGAGAGCACAGAGGAGTGG  
GCGGGGACCATGCGGGGACGCGGCTGGCGCTCCTGGCGCTGGTGTGCTGGCTGCCTGCGGAGAGCTGGCGCCGGC  
CCTGCGCTGCTACGTCTGTCCGGAGCCCACAGGAGTGTGGACTGTGTCACCATCGCCACCTGCACCACCAACG  
AAACCATGTGCAAGACCACACTCTACTCCCGGGAGATAGTGTACCCCTTCCAGGGGGACTCCACGGTGACCAAG  
TCCTGTGCCAGCAAGTGTAAAGCCCTCGGATGTGGATGGCATCGGCCAGACCCTGCCCCTGTCTGCTGCAATAC  
TGAGCTGTGCAATGTAGACGGGGCGCCCGCTCTGAACAGCCTCCACTGCGGGGGCCCTCACGCTCCTCCCACTCT  
TGAGCCTCCGACTGTAGAGTCCCCGCCACCCCATGGCCCTATGCGGCCAGCCCCGAATGCCTTGAAGAAGT  
GCCCCCTGCACCAGGAAAAAAAAAAAAAAAA

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Figure 28

MRGTRLALLALVLAACGELAPALRCYVCPEPTGVSDCVTIATCTTNETMCKTTLYSREIVYPFQGDSTVTKSCA  
SKCKPSDVDGIGQTLPVSCCNTELCNVDGAPALNSLHCGALTLLPLLSRL

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Figure 29

AAACTTGACGCCATGAAGATCCCGGTCCTTCCTGCCGTGGTGCTCCTCTCCCTCCTGGTGCTCCACTCTGCCCCA  
GGGAGCCACCCTGGGTGGTCCTGAGGAAGAAAGCACCATTGAGAATTATGCGTCACGACCCGAGGCCTTTAACA  
CCCCGTTCTTGAACATCGACAAATTGCGATCTGCGTTTAAGGCTGATGAGTTCCTGAACTGGCAGCCCTCTTT  
GAGTCTATCAAAAGGAAACTTCCTTTCCCTCAACTGGGATGCCTTTCCTAAGCTGAAGGACTGAGGAGCGCAAC  
TCCTGATGCCCAGTGACCATGACCTCCACTGGAAGAGGGGGCTAGCGTGAGCGCTGATTCTCAACCTACCATAA  
CTCTTTCCTGCCTCAGGAACTCCAATAAAACATTTTCATCCAA

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Figure 30

MKIPVLPVAVLLSLLVLHSAQGATLGGPEEEESTIENYASRPEAFNTPFLNIDKLRSFAKAEFLNWHALFESIK  
RKLPLFLNWDAFPCLKGLRSATPDAQ

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Figure 31

ATTCTCCTAGAGCATCTTTGGAAGCATGAGGCCACGATGCTGCATCTTGGCTCTTGTCTGCTGGATAACAGTCT  
TCCTCCTCCAGTGTTCAAAAGGAACCTACAGACGCTCCTGTTGGCTCAGGACTGTGGCTGTGCCAGCCGACACCC  
AGGTGTGGGAACAAGATCTACAACCCTTCAGAGCAGTGCTGTTATGATGATGCCATCTTATCCTTAAAGGAGAC  
CCGCCGCTGTGGCTCCACCTGCACCTTCTGGCCCTGCTTTGAGCTCTGCTGTCCCGAGTCTTTTGGCCCCCAGC  
AGAAGTTTCTTGTGAAGTTGAGGGTTCTGGGTATGAAGTCTCAGTGTCACTTATCTCCCATCTCCCGGAGCTGT  
ACCAGGAACAGGAGGCACGTCCTGTACCCATAAAAAACCCAGGCTCCACTGGCAGACGGCAGACAAGGGGAGAA  
GAGACGAAGCAGCTGGACATCGGAGACTACAGTTGAACTTCGGAGAGAAGCAACTTGACTTCAGAGGGATGGCT  
CAATGACATAGCTTTGGAGAGGAGCCAGCTGGGGATGGCCAGACTTCAGGGGAAGAATGCCTTCCTGCTTCAT  
CCCCTTTCAGCTCCCCTTCCCGCTGAGAGCCACTTTCATCGGCAATAAAATCCCCCACATTTACCATCT



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Figure 32

MRPRCCILALVCWITVFLQCSKGTDDAPVGSLWLCQPTPRCGNKIYNPSEQCCYDDAILSLKETRRCGSTCT  
FWPCFELCCPESFGFQQKFLVKLRVLGMKSQCHLSPIRSCTRNRRHVLYP

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Figure 33

GCGGCCGCTCGACCGGGCCCTGCGGGCGCGGGGCTGAAGGCGGAACCACGACGGGAGAGAGCACGGAGCCGG  
GAAGCCCCTGGGCGCCCGTTCGGAGGGCTATGGAGCAGCGGCCGCGGGGCTGCGCGCGGTGGCGGCGCGCTCC  
TCCTGGTGCTGCTGGGGGCCCCGGGCCCAGGGCGGCACTCGTAGCCCCAGGTGTGACTGTGCCGGTGAATTCCAC  
AAGAAGATTGGTCTGTTTTGTTGCAGAGGCTGCCAGCGGGGCACTACCTGAAGGCCCTTGACGGAGCCCTG  
CGGCAACTCCACCTGCCTTGTGTGTCCCCAAGACACCTTCTTGGCCTGGGAGAACCACCATAATTCTGAATGTG  
CCCCGTGCCAGGCCGTGTGATGAGCAGGCCCTCCCAGGTGGCGCTGGAGAACTGTTTACGAGTGGCCGACACCCGC  
TGTGGCTGTAAGCCAGGCTGGTTTGTGGAGTGCCAGGTGAGCCAATGTGTCAGCAGTTCACCCCTCTACTGCCA  
ACCATGCCTAGACTGCGGGGCCCTGCACCGCCACACACGGCTACTCTGTTCCCGCAGAGATACTGACTGTGGGA  
CCTGCCTGCCTGGCTTCTATGAACATGGCGATGGCTGCGTGTCTTCCCCACGAGCACCCCTGGGGAGCTGTCCA  
GAGCGCTGTGCCGCTGTCTGTGGCTGGAGGCAGATGTTCTGGGTCCAGGTGCTCCTGGCTGGCCTTGTGGTCCC  
CCTCCTGCTTGGGGCCACCCTGACCTACACATACCGCCACTGCTGGCCTCACAAGCCCCTGGTTACTGCAGATG  
AAGCTGGGATGGAGGCTCTGACCCACCACCGGCCACCCATCTGTACCCCTTGGACAGCGCCACACCCCTCTA  
GCACCTCCTGACAGCAGTGAGAAGATCTGCACCGTCCAGTTGGTGGGTAACAGCTGGACCCCTGGCTACCCCGA  
GACCCAGGAGGGCTCTGCCCCAGGTGACATGGTCTTGGGACCAGTTGCCAGCAGAGCTCTTGGCCCCGCTG  
CTGCGCCACACTCTCGCCAGAGTCCCCAGCGGGCTCGCCAGCCATGATGCTGCAGCGGGCCCGCAGCTCTAC  
GACGTGATGGACGCGGTCCCAGCGCGGCGTGAAGGAGTTCTGCGCACGCTGGGGCTGCGCGAGGCAGAGAT  
CGAAGCCGTGGAGGTGGAGATCGGCCGCTTCCGAGACCAGCAGTACGAGATGCTCAAGCGCTGGCGCCAGCAGC  
AGCCCGCGGGCCTCGGAGCCGTTTACGCGGCCCTGGAGCGCATGGGGCTGGACGGCTGCGTGAAGACTTGCGC  
AGCCGCTGACAGCGCGGCCCTGACACGGCGCCCACTTGCCACCTAGGCGCTCTGGTGGCCCTTGCAAGCCCC  
TAAGTACGGTTACTTATGCGTGTAGACATTTATGTCACTTATTAAGCCGCTGGCACGGCCCTGCGTAGCAGCA  
CCAGCCGGCCCCACCCCTGCTCGCCCTATCGCTCCAGCCAAGGCGAAGAAGCACGAACGAATGTGAGAGGGG  
GTGAAGACATTTCTCAACTTCTCGGCCGGAGTTTGGCTGAGATCGCGGTATTAAATCTGTGAAAGAAAACAAAA  
AAAAAAAAAAAAAAAAAAGTCGACGCGGCCGC

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Figure 34

MEQRPRGCAAVAAALLLVLLGARAQGGTRSPRCD CAGDFHKKIGLFCCRGCPAGHYLKAPCTEPCGNSTCLVCP  
QDTFLAWENHHNSECARCQACDEQASQVALENC SAVADTRCGCKPGWFVECVSQCVSSSPFYCQPCLD CGALH  
RHTRLLCSRRDTCGTCLPGFYEHGDGCVSCPTSTLGSCPERCAAVCGWRQMFVQVLLAGLVVPLLLGATLTY  
TYRHCWPHKPLVTAD EAGMEALTPPPATHLSPLDSAHTLLAPPDSSEKICTVQLVGN SWTPGYPETQEALCPQV  
TWSWDQLPSRALGPAAAPTLSPE SPAGSPAMMLQPGPQLYDVMDAVPARRWKEFVRTLGLREA EIEAVEVEIGR  
FRDQQYEMLKRRWQQQPAGLGAVYAA LERMGLDGCVEDLRSRLQ RGP

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Figure 35

CAACAGAAGCCAAGAAGGAAGCCGTCTATCTTGTGGCGATCATGTATAAGCTGGCCTCCTGCTGTTTGCTTTTC  
ACAGGATTCTTAAATCCTCTCTTATCTCTTCTCTCCTTGACTCCAGGGAAATATCCTTTCAACTCTCAGCACC  
TCATGAAGACGCGCGCTTAACTCCGGAGGAGCTAGAAAGAGCTTCCCTTCTACAGATATTGCCAGAGATGCTGG  
GTGCAGAAAGAGGGGATATTCTCAGGAAAGCAGACTCAAGTACCAACATTTTAAACCAAGAGGAAATTTGAGA  
AAGTTTCAGGATTTCTCTGGACAAGATCCTAACATTTTACTGAGTCATCTTTTGGCCAGAATCTGGAAACCATA  
CAAGAAACGTGAGACTCCTGATTGCTTCTGGAAATACTGTGCTGAAGTGAAATAAGCATCTGTTAGTCAGCTC  
AGAAACACCCATCTTAGAATATGAAAAATAACACAATGCTTGATTGAAAAACAGTGTGGAGAAAAACTAGGCAA  
ACTACACCCTGTTTCATTGTTACCTGGAAAAATAATCCTCTATGTTTTGCACAAAAAAAAAAAAAAAAA

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Figure 36

MYKLASCCLLFTGFLNPLLSLPLDSREISFQLSAPHEDARLTPEELERASLLQILPEMLGAERGDILRKADSS  
TNIFNPRGNLRKFQDFSGQDPNILLSHLLARIWKPYKKRET?DCFWKYCV

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Figure 37

AGGCGGGCAGCAGCTGCAGGCTGACCTTGCAGETTGGCGGAATGGACTGGCCTCACAACCTGCTGTTTCTTCTT  
ACCATTTCCATCTTCTGGGGCTGGGCCAGCCCAGGAGCCCCAAAAGCAAGAGGAAGGGGCAAGGGCGGCCTGG  
GCCCCTGGCCCCTGGCCCTCACCAGGTGCCACTGGACCTGGTGTACCGGATGAAACCGTATGCCCGCATGGAGG  
AGTATGAGAGGAACATCGAGGAGATGGTGGCCCAGCTGAGGAACAGCTCAGAGCTGGCCCAGAGAAAGTGTGAG  
GTCAACTTGCAGCTGTGGATGTCCAACAAGAGGAGCCTGTCTCCCTGGGGCTACAGCATCAACCACGACCCCAG  
CCGTATCCCCGTGGACCTGCCGGAGGCACGGTGCCTGTGTCTGGGCTGTGTGAACCCCTTCACCATGCAGGAGG  
ACCGCAGCATGGTGAGCGTGCCGGTGTTCAGCCAGGTTCCGTGTGCGCCGCCGCTCTGCCCGCCACCGCCCCGC  
ACAGGGCCTTGCCGCCAGCGCGCAGTCATGGAGACCATCGCTGTGGGCTGCACCTGCATCTTCTGAATCACCTG  
GCCCAGAAGCCAGGCCAGCAGCCGAGACCATCCTCCTTGACCTTTGTGCCAAGAAAGGCCTATGAAAAGTAA  
ACACTGACTTTTGAAAGCAAG

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Figure 38

MDWPHNLLFLLTISIFLGLGQPRSPKSKRKQGGRPGPLAPGPHQVPLDLVSRMKPYARMEEYERNIEEMVAQLR  
NSSELAQRKCEVNLQLWMSNKRSLSPWGYSINEDPSRIPVDLPEARCLCLGCVNPFMTQEDRSMVSVPVFSQVP  
VRRRLCPEPPRTGPCRQRAVMETIavgctcIF

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Figure 39

TGGACTTCTCTGGACCACAGTCCTCTGCCAGACCCCTGCCAGACCCCAAGTCCACCATGATCCATCTGGGTCACA  
TCCTCTTCCTGCTTTTGCTCCCAGTGGCTGCAGCTCAGACGACTCCAGGAGAGAGATCATCACTCCCTGCCTTT  
TACCCTGGCACTTCAGGCTCTTGTTCCGGATGTGGGTCCCTCTCTCTGCCGCTCCTGGCAGGCCTCGTGGCTGC  
TGATGCGGTGGCATCGCTGCTCATCGTGGGGGCGGTGTTCCCTGTGCGCACGCCACGCCGAGCCCCGCCAAG  
ATGGCAAAGTCTACATCAACATGCCAGGCAGGGGCTGACCCCTCCTGCAGCTTGGACCTTTGACTTCTGACCCTC  
TCATCCTGGATGGTGTGTGGTGGCACAGGAACCCCGCCCCAACTTTGGATTGTAATAAAACAATTGAAACAC  
CA



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Figure 40

MIHLGHILFLLLLPVAAAQTT?GERSSLPAFYPGTSGSCSGCGLSLPLLAGLVAAADAVASLLIVGAVFLCAR?  
RRSPAQDGKVYINMPGRG

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Figure 41

AATATATCATCTATTTATCATTAAATCAATAATGTATTCTTTTATTCCAATAACATTTGGGTTTTGGGATTTTAA  
TTTTCAAACACAGCAGATGACATTTTTCTGTCACTATTATTATTGTTGGTATGTGAAGCTATTTGGAGATCC  
AATTCAGGAAGCAACACATTGGAGAATGGCTACTTTCTATCAAGAAATAAAGAGAACCACAGTCAACCCACACA  
ATCATCTTTAGAAGACAGTGTGACTCCTACCAAAGCTGTCAAPACCACAGGCAAGGGCATAGTTAAAGGACGGA  
ATCTTGACTCAAGAGGGTTAATTCTTGGTGCTGAAGCCTGGGGCAGGGGTGTAAAGAAAAACACTTAGATTCAA  
TGATTGTAAATTTAAGGCAAATACACATATTAGTATTACCTTAGTGTAATGTATCCCTGTCATATATACAATAA  
GGTGAAATTATAAGTACCCTATGCAGTTGGCTGGACAGTTCTAAATTGGACTTTATTAATTTTTAAATCAGTA  
ACTGATTTATCACTGGCTATGTGCTTAGATCTACAGGAGATCATATAATTTGATACAAATAAAGAAAAAGTGT  
CTCTCCCTTACAGATTGACATTTTAAATGCGATACAGTTAGAATAGGAAATATGACATTAGAAAGGAAGAAT  
GACAGGGAGAAAGGAAGAAGGGAAAATGTTGCCAAGGAAAAA

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Figure 42

MTFFLSLLLLLVCEAIWRSNSGSNTLENGYFLSRNKENHSQPTQSSLEDSVTPTKAVKTTGKGIVKGRNLDSRG  
LILGAEAWGRGVKKNT

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Figure 43

GGAGGCGGAGGCGCGGCGAGCCGGGCGGAGCAGTGAGGGCCCTAGCGGGGCGGAGCGGGGCGGCGGCGCCCT  
AAGCCATTCTGAAGTCATGGGCTGGCCAGGACATTGGTGACCCGCCAATCCGGTATGGAGGACTGGAAGCCCA  
GCCCCCTCATCAAGCCCTTTGGGGCTCGGAAGAAGCGGAGCTGGTACCTTACCTGGAAGTATAAACTGACAAAC  
CAGCGGGCCCTGCGGAGATTCTGTGACAGAGGGGCGGTGCTTTTCTGCTGGTGACTGTCTATTGTCAATATCAA  
GTTGATCCTGGAGACTCGGCGAGCCATCAGTGAAGCCAATGAAGACCCAGAGCCAGAGCAAGACTATGATGAG  
GCCCCAGGCGGCTGGAGCCCCACGGGCGCAGAGGCAGTGGTCCCCGGCGGGTCTGGACCTAGAGGTGTATTTC  
AAGTCGCAGCAAAGTATATGTGGCAGTGGATGGCACCACGGTGTGGAGGATGAGGCGCGGAGCAGGGCCGGG  
GCATCCATGTCTATTGTCCTCAACCAGGCCACGGGCCACGTGATGGCAAAACGTGTGTTTGACACGTACTCACCT  
CATGAGGATGAGGCCATGGTGCTATTCTCAACATGGTAGCGCCCGGCGGAGTGCTCATCTGCACTGTCAAGGA  
TGAGGGCTCCTTCCACCTCAAGGACACAGCCAAGGCTCTGCTGAGGAGCCTGGGCAGCCAGGCTGGCCCTGCCC  
TGGGCTGGAGGACACATGGGCTTCTGGGACGAAAAGGAGGTCTGTCTTCGGGAGAAACATTCTAAGTCA  
CCTGCCCTCTCTTCTGGGGGAGCCAGTCTCTGCTGAAGACAGATGTGCCATTGAGCTCAGCAGAAGAGGCAGA  
GTGCCACTGGGCAGACACAGAGCTGAACCGTGGCGCGGCGCTTCTGCAGCAAAGTTGAGGGCTATGGAAGTG  
TATGCAGCTGCAAGGACCCACACCCATCGAGTTTACGGCTGACCCACTCCCAGACAACAAGGTCTCAATGTG  
CCTGTGGCTGTCTTGCAGGGAACCGACCCAATTACCTGTACAGGATGCTGCGCTCTCTGCTTTCAGCCAGGG  
GGTGTCTCTCAGATGATAACAGTTTTTATTGACGGCTACTATGAGGAACCCATGGATGTGGTGCCACTGTTTG  
GTCTGAGGGGCATCCAGCATACTCCCATCAGCATCAAGAATGCCCGCGTGTCTCAGCACTACAAGGCCAGCCTC  
ACTGCCACTTTCAACCTGTTTTCCGGAGGCCAAGTTTTGCTGTGGTTCTGGAAGAGGACCTGGACATTGCTGTGGA  
TTTTTTTCAAGTTTCTGAGCCAATCCATCCACCTACTGGAGGAGGATGACAGCCTGTACTGCATCTCTGCTGGA  
ATGACCAGGGGTATGAACACACGGCTGAGGACCCAGCACTACTGTACCGTGTGGAGACCATGCCTGGGCTGGGC  
TGGGTGCTCAGGAGGTCTTGTACAAGGAGGAGCTTGAGCCCAAGTGGCCTACACCGGAAAAGCTCTGGGATTG  
GGACATGTGGATGCGGATGCCTGAACAACGCCGGGCGGAGAGTGCATCATCCCTGACGTTTCCCGATCCTACC  
ACTTTGGCATCGTCGGCCTCAACATGAATGGCTACTTTTACGAGGCCTACTTCAAGAAGCACAAGTTCAACACG  
GTTCCAGGTGTCCAGCTCAGGAATGTGGACAGTCTGAAGAAAGAAGCTTATGAAGTGGAAGTTCACAGGCTGCT  
CAGTGAGGCTGAGGTTCTGGACCAACAGCAAGAACCCTTGTGAAGACTCTTCTGCCAGACACAGAGGGCCACA  
CCTACGTGGCCTTTATTGGAATGGAGAAAGATGATGACTTCAACACCTGGACCCAGCTTGCCAAGTGCCTCCAT  
ATCTGGGACCTGGATGTGCGTGGCAACCATCGGGGCTGTGGAGATTGTTTCGGAAGAAGAACCCTTCTGCTGGT  
GGTGGGGGTCCCCGCTTCCCCCTACTCAGTGAAGAAGCCACCCTCAGTCACCCCAATTTTCTGGAGCCACCCC  
CAAAGGAGGAGGGAGCCCCAGGAGCCCCAGAACAGACATGAGACCTCCTCCAGGACCTGCGGGGCTGGGTACT  
GTGTACCCCGAGGCTGGCTAGCCCTTCCCTCCATCCTGTAGGATTTTGTAGATGCTGGTAGGGGCTGGGGCTAC  
CTTGTTTTTAACATGAGACTTAATTACTAATCCAAGGGGAGGGTTCCCTGCTCCAACACCCCGTCTCTGAGT  
TAAAAGTCTATTTTACTTCTTGTGGAGAAGGGCAGGAGAGTACCTGGGAATCATTACGATCCCTAGCAG  
CTCATCTGCCCCTTGAATACCCTCACTTTCAGGCTGGCTCAGAATCTAACCTATTTATTGACTGTCTGAG  
GGCCTTGAACAGGCCGAACCTGGAGGGCCTGGATTTCTTTTGGGCTGGAATGCTGCCCTGAGGGTGGGGCT  
GGCTCTTACTCAGGAACTGCTGTGCCCAACCCATGGACAGGCCCAGCTGGGGCCCATGCTGACACAGACTC  
ACTCAGAGACCCCTAGACACTGGACCAGGCTCTCTCAGCCTTCTCTTGTCCAGATTTCCAAGCTGGATAA  
GTTGGTCATTGATTAAAAAAGGAGAAGCCCTCTGGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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Figure 44

MDDWKPSPLIKPFGARKKRSWYLTWKYKLTNQRALRRFCQTGAVLFLLVTIVIVNIKLILDTRRAISEANEDPEP  
EQDYDEALGRLEPPRRRGSGPRRVLDVEVYSSRSKVYVAVDGTTVLEDEAREQGRGIHVIVLNQATGHVMAKRV  
FOTYSPHEDEAMVLFNLMVAPGRVLICTVKDEGSFHLKDTAKALLRSLGSQAGPALGWRDTWAFVGRKGGPVFG  
EKHSKSPALSSWGDVPVLLKTDVPLSSAEAECHWADTELNRRRRRFCSKVEGYGSVCCKDPTPIEFSPDPLPD  
NKVLNVPVAVIAGNRPNLYRMLRSLLSAQGVSPQMITVFTDGYEPMDDVVALFGLRGIQHTPISIKNARVSQ  
HYKASLTATFNLFPFAKFAVVLEEDLDIAVDFSFLSQSIHLLLEEDDSLYCISAWNDOGYEHTAEDPALLYRVE  
TMPGLGWVLRRLYKEELEPKWPTPEKLWDWDMWMMRMPEQRRGRECIIPDVSRSYHFGIVGLNMNGYFHEAYFK  
KHKFNTVPGVQLRNVDLKKAEYEVVHRLLEAEVLHDSKNPCEDSFLPDTEGHTYVAFIRMEKDDDDFTTWTQ  
LAKCLHIWDLVVRGNHRGLWRLFRKKNHFLVVGVPASPSVKKPPSVTPIFLEPPKKEGAPGAPEQT

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Figure 45

GCGGAGCCGGCGCCGGCTGCGCAGAGGAGCCGCTCTCGCCGCGCCACCTCGGCTGGGAGCCCACGAGGCTGCC  
GCATCCTGCCCTCGGAACAATGGGACTCGGCGCGGAGGTGCTTGGGCGCGCTGCTCCTGGGGACGCTGCAGG  
TGCTAGCGCTGCTGGGGGCGCCCATGAAAGCGCAGCCATGGCGGCATCTGCAAACATAGAGAATTCTGGGCTT  
CCACACAACCTCCAGTGCTAACTCAACAGAGACTCTCCAACATGTGCCTTCTGACCATACAAATGAAACTTCCAA  
CAGTACTGTGAAACCACCAACTTCAGTTGCCTCAGACTCCAGTAATACAACGGTCACCACCATGAAACCTACAG  
CGGCATCTAATAACAACAACACCAGGGATGGTCTCAACAAATATGACTTCTACCACCTTAAAGTCTACACCCAAA  
ACAACAAGTGTTCACAGAACACATCTCAGATATCAACATCCACAATGACCGTAACCCACAATAGTTCAGTGAC  
ATCTGCTGCTTCATCAGTAACAATCACAACAATATGCATTCTGAAGCAAAGAAAGGATCAAAATTTGATACTG  
GGAGCTTTGTTGGTGGTATTGTATTAACGCTGGGAGTTTTATCTATTCTTTACATTGGATGCAAAATGTATTAC  
TCAAGAAGAGGCATTCCGGTATCGAACCATAGATGAACATGATGCCATCATTTAAGGAAATCCATGGACCAAGGA  
TGGAATACAGATTGATGCTGCCCTATCAATTAATTTGGTTTATTAATAGTTTAAACAATATTCTCTTTTGA  
AAATAGTATAAACAGGCCATGCATATAATGTACAGTGTATTACGTAAATATGTAAAGATTCTTCAAGGTAACAA  
GGGTTTGGGTTTTGAAATAAACATCTGGATCTTATAGACCGTTCATACAATGGTTTTAGCAAGTTCATAGTAAG  
ACAACAAGTCCTATCTTTTTTTTTTGGCTGGGGTGGGGGCATTGGTCACATATGACCAGTAATTGAAAGACGT  
CATCACTGAAAGACAGAATGCCATCTGGGCATACAAATAAGAAGTTTGTACAGCACTCAGGATTTTGGGTATC  
TTTTGTAGCTCACATAAAGAACTTCAGTGCTTTTCAGAGCTGGATATATCTTAATTACTAATGCCACACAGAAA  
TTATACAATCAAACCTAGATCTGAAGCATAATTTAAGAAAAACATCAACATTTTTTGTGCTTTAACTGTAGTAG  
TTGGTCTAGAAACAAAATACTCC

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Figure 46

MGLGARGAWAALLLGT LQVLALLGA AHESAAMAASANIENSG LPHNSSANSTETLQHVPSDHTNETSNSTVKPP  
TSVASDSSNTTVTTMKPTAASNTTTPGMVSTNMTSTTLKSTPKTTSVSQNTSQISTSTMTVTHNSSVTSAASSV  
TITTTMHSEAKKGSKFDTGSFVGGIVLT LGVLSILYIGCKMYYSRAGIRYRTIDEHDAII

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Figure 47

GGGAGAGAGGATAAATAGCAGCGTGGCTTCCCTGGCTCCTCTCTGCATCCTTCCCGACCTTCCCAGCAATATGC  
ATCTTGACGCTCTGGTCGGCTCCTGCTCCCTCCTTCTGCTACTGGGGGCCCTGTCTGGATGGGCGGCCAGCGAT  
GACCCCATTTGAGAAGGTCATTGAAGGGATCAACCGAGGGCTGAGCAATGCAGAGAGAGAGGTGGGCAAGGCCCT  
GGATGGCATCAACAGTGAATCACGCATGCCGGAAGGGAAGTGGAGAAGGTTTCAACGGACTTAGCAACATGG  
GGAGCCACACCGGCAAGGAGTTGGACAAAGGCGTCCAGGGGCTCAACCACGGCATGGACAAGGTTGCCCATGAG  
ATCAACCATGGTATTGGACAAGCAGGAAAGGAAGCAGAGAAGCTTGGCCATGGGGTCAACAACGCTGCTGGACA  
GGCCGGAAGGAAGCAGACAAAGCGGTCCAAGGGTTCACACTGGGGTCCACCAGGCTGGGAAGGAAGCAGAGA  
AACTTGGCCAAGGGGTCAACCATGCTGCTGACCAGGCTGGAAAGGAAGTGGAGAAGCTTGGCCAAGGTGCCCCAC  
CATGCTGCTGGCCAGGCCGGGAAGGAGCTGCAGAATGCTCATAATGGGGTCAACCAAGCCAGCAAGGAGGCCAA  
CCAGCTGCTGAATGGCAACCATCAAAGCGGATCTTCCAGCCATCAAGGAGGGGCCACAACCACGCCGTTAGCCT  
CTGGGGCCTCAGTCAACACGCCCTTTCATCAACCTTCCCGCCCTGTGGAGGAGCGTCGCCAACATCATGCCCTAA  
ACTGGCATCCGGCCTTGCTGGGAGAATAATGTCGCCGTTGTACATCAGCTGACATGACCTGGAGGGGTTGGGG  
GTGGGGGACAGGTTTCTGAAATCCCTGAAGGGGTTGTAAGGATTTGTGAATAAACTTGATACACCA



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Figure 48

MHLARLVGSCSLLLLLGALSGWAASDDFIEKVIEGINRGLSNAEREVGKALDGINSGITHAGREVEKVFNGLSN  
MGSHTGKELDKGVQGLNHGMDKVAHEINHGIGQAGKEAEKLGHGYNNAAGQAGKEADKAVQGFHTGVHQAGKEA  
EKLGGQGVNHAADQAGKEVEKLGQGAHHAACQAGKELQNAHNGVNOASKEANQLNGNHQSGSSSHQGGATTTPL  
ASGASVNTPFINLPALWRSVANIMP

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Figure 49

CAGCCGGGTCCCAAGCCTGTGCCTGAGCCTGAGCCTGAGCCTGAGCCCGAGCCGGGAGCCGGTGC GGGGGCTC  
CGGGCTGTGGGACCGCTGGGCCCCAGCGATGGCGACCCTGTGGGGAGGCCTTCTTCGGCTTGGCTCCTTGCTC  
AGCCTGTGCTGCTGGCGCTTTCCGTGCTGCTGCTGGCGCAGCTGTCAGACGCCCAAGAATTTTCGAGGATGT  
CAGATGTAAATGTATCTGCCCTCCCTATAAAGAAAATTCTGGGCATATTTATAATAAGAACATATCTCAGAAAG  
ATTGTGATTGCCCTTCATGTTGTGGAGCCCATGCCTGTGCGGGGGCCTGATGTAGAAGCATACTGTCTACGCTGT  
GAATGCAAATATGAAGAAAGAAGCTCTGTCACAATCAAGGTTACCATTATAATTTATCTCTCCATTTTGGGCCT  
TCTACTTCTGTACATGGTATATCTTACTCTGGTTGAGCCCATACTGAAGAGGCGCCTCTTTGGACATGCACAGT  
TGATACAGAGTGATGATGATATTGGGGATCACCAGCCTTTTGCAAATGCACACGATGTGCTAGCCCGCTCCCGC  
AGTCGAGCCAACGTGCTGAACAAGGTAGAATATGCACAGCAGCGCTGGAAGCTTCAAGTCCAAGAGCAGCGAAA  
GTCTGTCTTTGACCGGCATGTTGTCTCAGCTAAATTGGGAATTGAATTCAAGGTGACTAGAAAGAAACAGGCAG  
ACAACTGGAAGAAGTACTGAGGTTTTGTGCTGGGTTTCATTTTAATACCTTGTGATTTTACCAACTGTTGCTGG  
AAGATTCAAAAGTGAAGCAAAAAGTCTGCTGATTTTTTTTTCTGTTAACGTAATAATAGAGACATTTTTAAA  
AGCACACAGCTCAAAGTCAGCCAATAAGTCTTTTCCTATTTGTGACTTTTACTAATAAAAAATAAATCTGCCTGT  
AAATTATCTTGAAGTCCTTTACCTGGAACAAGCACTCTCTTTTACCACATAGTTTAACTTGACTTTCAAGA  
TAATTTTCAGGGTTTTTGTGTTGTTGTTTTGTTGTTGTTTGGTGGGAGAGGGGAGGGATGCCTGGGAA  
GTGGTTAACAACTTTTTCAAGTCACTTTACTAAACAAACTTTTGTAATAGACCTTACCTTCTATTTTCGAGT  
TTCATTTATATTTTGCAGTGTAGCCAGCCTCATCAAAGAGCTGACTTACTCATTGACTTTTGCAGTACTGTA  
TTATCTGGGTATCTGCTGTGCTGCACCTCATGGTAAACGGGATCTAAAATGCCTGGTGGCTTTTACAAAAAG  
CAGATTTTCTTCATGTAAGTGTGATGTCTGATGCAATGCATCCTAGAACAAGTGGCCATTTGCTAGTTTACTCT  
AAAGACTAAACATAGTCTTGGTGTGTGTTGCTTACTCATCTTCTAGTACCTTTAAGGACAAATCCTAAGGACT  
TGGACACTTGCAATAAAGAAATTTATTTTAAACCCAGCCTCCCTGGATTGATAATATATACACATTTGTGAG  
CATTTCCGGTCGTGGTGAGAGGCAGCTGTTGAGCTCCAATATGTGCAGCTTTGAACTAGGGCTGGGGTTGTGG  
GTGCCTCTTCTGAAAGGTCTAACCATTATTGGATAACTGGCTTTTTTCTTCCTATGTCTCTTTGGAATGTAAC  
ATAAAAAATAATTTTGAACATCAA

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Figure 50

MATLWGGLRLGSLLSLSCLALSVLLLAQLSDAAKNFEDVRCKCICPPYKENS  
GHIYNKNISQKDCDCLHVVEP  
MPVRGPDVEAYCLRCECKYEERSSVTIKVTIIYLSILGLLLLYMYLTLVEPILKRRLFGHAQLIQSDDDIGD  
HQPFAHAHDVLRSSRANVLNKVEYAQQRWKLQVQEQRKSVFDRHVVL

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Figure 51

GGACAACCGTTGCTGGGTGTCCAGGGCCTGAGGCAGGACGGTACTCCGCTGACACCTTCCCTTTCGGCCTTGA  
GGTTCACGCTGGTGGCCCCAGGACGTTCCGGTCGCATGGCAGAGTGCTACGGACGACGCCTATGAAGCCCTT  
AGTCCTTCTAGTTGCGCTTTTGCTATGGCCTTCGTCTGTGCCGGCTTATCCGAGCATAACTGTGACACCTGATG  
AAGAGCAAACTTGAATCATTATATACAAGTTTTAGAGAACCTAGTACGAAGTGTTCCCTCTGGGGAGCCAGGT  
CGTGAGAAAAATCTAACTCTCCAAAACATGTTTATTCTATAGCATCAAAGGGATCAAAATTTAAGGAGCTAGT  
TACACATGGAGACGCTTCAACTGAGAATGATGTTTAAACCAATCCTATCAGTGAAGAACTACAACCTTCCCTA  
CAGGAGGCTTACACCCGGAATAGGAAAGAAAAACACACGGAAAGTACCCCATTTCTGGTCGATCAAACCAAC  
AATGTTTCCATTGTTTTGCATGCAGAGGAACCTTATATTGAAAATGAAGAGCCAGAGCCAGAGCCGGAGCCAGC  
TGCAAAACAACTGAGGCACCAAGAATGTTGCCAGTTGTTACTGAATCATCTACAAGTCCATATGTTACCTCAT  
ACAAGTCACCTGTCACCACTTTAGATAAGAGCACTGGCATTGAGATCTCTACAGAATCAGAAGATGTTCTCAG  
CTCTCAGGTGAACTGCGATAGAAAAACCCGAAGAGTTTGGAAAGCACCCAGAGAGTTGGAATAATGATGACAT  
TTTGAAAAAATTTTAGATATTAATTCACAAGTGCAACAGGCACCTTCTAGTGACACCAGCAACCCAGCATATA  
GAGAAGATATTGAAGCCTCTAAAGATCACCTAAAACGAAGCCTTGCTCTAGCAGCAGCAGCAGAACATAAAATTA  
AAAACAATGTATAAGTCCCAGTTATTGCCAGTAGGACGAACAAGTAATAAAATTGATGACATCGAACTGTTAT  
TAACATGCTGTGTAATTCTAGATCTAACTCTATGAATATTTAGATATTAAATGTGTTCCACCAGAGATGAGAG  
AAAAAGCTGCTACAGTATTCAATACATTAATAAATATGTGTAGATCAAGGAGAGTCACAGCCTTATTAAGTT  
TATTAAACAATAATATAAAATTTTAAACCTACTTGATATTCCATAACAAAGCTGATTTAAGCAAACTGCATTT  
TTTCACAGGAGAAATAATCATATTCGTAATTTCAAAGTTGTATAAAATATTTTCTATTGTAGTTCAAATGTG  
CCAACATCTTTATGTGTCATGTGTTATGAACAATTTTCATATGCACTAAAAACCTAATTTAAATAAAATTTTG  
GTTCAGGAAAAAA

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Figure 52

MKPLVLLVALLWPSSVPAYPSITVTPDEEQNLNHYIQVLENLVRSVPSGEPGREKKSNSPKHVYSIASKGSKF  
KELVTHGDASTENDVLNPISEETTTFTGGFTPEIGKKKHTESTPFWSIKPNNVSIVLHAEOPYIENEPEPE  
PEPAAKQTEAPRMLPVVTESSSTSPYVTSYKSPVTTLOKSTGIEISTESEDVPQLSGETAIEKPEEFGKHPESWN  
NDDILKKILDINSQVQQALLSDTSNPAYREDIEASKDHLKRSLALAAAAEHKLKTMYSQLLPVGRTSNKIDDI  
ETVINMLCNSRSKLYEYLDIKVPPPEMREKAATVENTLKNMCRSRRVTALLKVY

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Figure 53

AGTGACAATCTCAGAGCAGCTTCTACACCACAGCCATTTCCAGCATGAAGATCACTGGGGGTCTCCTTCTGCTC  
TGTACAGTGGTCTATTTCTGTAGCAGCTCAGAAGCTGCTAGTCTGTCTCCAAAAAAGTGGACTGCAGCATTTA  
CAAGAAGTATCCAGTGGTGGCCATCCCCTGCCCCATCACATACCTACCAGTTTGTGGTTCTGACTACATCACCT  
ATGGGAATGAATGTCACCTTGTGTACCGAGAGCTTGAAAAGTAATGGAAGAGTTCAGTTTCTTCACGATGGAAGT  
TGCTAAATTCTCCATGGACATAGAGAGAAAGGAATGATATTCTCATCATCATCTTCATCATCCCAGGCTCTGAC  
TGAGTTTCTTTCAGTTTTACTGATGTTCTGGGTGGGGACAGAGCCAGATTCAGAGTAATCTTGACTGAATGGA  
GAAAGTTTCTGTGCTACCCCTACAAACCCATGCCTCACTGACAGACCAGCATTTTTTTTTTAACACGTCAATAA  
AAAAATAATCTCCAGA

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Figure 54

MKITGGLLLCTVVYFCSSEASLSPKKVDCSIYKKYPVVAIPCPITYLPVCGSDYITYGNECHLCTESLKSN  
GRVQFLHDGSC

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Figure 55

CGACGATGCTACGCGCGCCCGGCTGCCTCCTCCGGACCTCCGTAGCGCCTGCCGCGGCCCTGGCTGCGGCGCTG  
CTCTCGTGGCTTGGCGGCTGCTCTCTTCTAGAGCCGAGGGACCCGGTGGCCTCGTCCGCTCAGCCCCCTATTTCCG  
CACCAAGACTCGCTACGAGGATGTCAACCCCGTGCTATTGTGCGGCCCGGAGGCTCCGTGGCGGGACCCCTGAGC  
TGCTGGAGGGGACCTGCACCCCGGTGCAGCTGGTCCGCCCTATTGCCACGGCACCCTGCTACCCACGGTCAAA  
CAGATCCGCAAGCTGAGGCAGCTGCACGGGTGCTGCAGGCCCGCGGGTCCAGGGATGGCGGGGCTAGTAGTAC  
CGGCAGCCCGGACCTGGGTGCAGCGCTGGCCGACTGGCCTTTGTGGTACGCGGACTGGATGGACGGGCAGCTAG  
TAGAGAAGGGACGGCAGGATATGCGACAGCTGGCGCTGCGTCTGGCCTCGCTCTTCCCGGCCCTTTTCAGCCGT  
GAGAAGTACGGCCCGCTGCGGCTCATCACCAGTTCCAAGCACCCTGCATGGATAGCAGCGCCGCTTCTCTGCA  
GGGGCTGTGGCAGCACTACCACCTGGCTTGGCCGCCCGGACGTCCGAGATATGGAGTTTGGACCTCCAACA  
GTTAATGATAAACTAATGAGATTTTTGATCACTGTGAGAAGTTTTTAAGTGAAGTAGAAAAAATGCTACAGC  
TCTTTATCAGTGGGAAGCCTTCAAACTGGACCAGAAATGCAGAACATTTTAAAAAAGTTGCAGCTACTTTGC  
AAGTGCCAGTAAATGATTTAAATGCAGATTTAATCAAGTAGCCTTTTTCACCTGTTCAATTTGACCTGGCAATT  
AAAGGTGTTAAATCTCTTGGTGTGATGTTTTGACATAGATGATGCAAGGTATTAGAATATTTAAATGATCT  
GAAACAATATTGGAAGAGGATATGGGTATACTATTAACAGTCGATCCAGCTGCACCTTGTTCAGGATATCT  
TTCAGCACTTGGACAAAGCAGTTGAACAGAAACAAAGGTCTCAGCCAATTTCTTCTCCAGTCATCTCCAGTTT  
GGTCATGCAGAGACTCTTCTTCCACTGCTTTCTCTCATGGGCTACTTCAAAGACAAGGAACCCCTAACAGCGTA  
CAATTACAAAAACAAATGCATCGGAAGTTCCGAAGTGGTCTCATTGTACCTTATGCCTCGAACCTGATATTTG  
TGCTTTACCACTGTGAAAATGCTAAGACTCCTAAAGAACAATTCCGAGTGCAGATGTTATTAATGAAAAGGTG  
TTACCTTTGGCTTACTCACAAGAACTGTTTCATTTTATGAGATCTGAAGAACCCTACAGGACATCTTCA  
GAGTGTCAAACCAGTGAAGAATGTGAATTAGCAAGGGCTAACAGTACATCTGATGAAGTATGAGTAACTGAAG  
AACATTTTTAATCTTTAGGAATCTGCAATGAGTGATTACATGCTTGTAAATAGGTAGGCAATTTCTTGATTACA  
GGAAGCTTTTATATTACTTGAGTATTTCTGTCTTTTCACAGAAAAACATTGGGTTTCTCTCTGGGTTTGGACAT  
GAAATGTAAGAAAAGATTTTTCACTGGAGCAGCTCTCTTAAGGAGAAACAAATCTATTTAGAGAAACAGCTGGC  
CCTGCAAATGTTTACAGAAATGAAATCTTCTTCTACTTATATAAGAAATCTCACACTGAGATAGAAATTTGTGATTT  
CATAATAACACTTGAAAAGTGTGGAGTAACAAAATATCTCAGTTGGACCATCTTAACTTGATTGAAGTGTCT  
AGGAACCTTACAGATTGTTCTGCAGTTCTCTCTTCTTTCTCAGGTAGGACAGCTCTAGCATTCTTCTTAATCA  
GGAATATTGTGGTAAGCTGGGAGTATCACTCTGGAAGAAAGTAACATCTCCAGATGAGAATTTGAAACAAGAAA  
CAGAGTGTGTGAAAAGGACACCTTCACTGAAGCAAGTCGGAAAGTACAATGAAAAATAATATTTTTGGTATTTA  
TTTATGAAATATTTGAACATTTTTTCAATAATTCCTTTTTTACTTCTAGGAAGTCTCAAAGACCATCTTAAATT  
ATTATATGTTTGGACAATTAGCAACAAGTCAGATAGTTAGAATCGAAGTTTTTCAAATCCATTGCTTAGCTAAC  
TTTTTCATTCTGTCACTTGGCTTCGATTTTTATATTTTCTATTATATGAAATGTATCTTTTGGTTGTTTGATT  
TTTCTTTCTTTCTTTGTAATAGTTCTGAGTTCTGTCAAATGCCGTGAAAGTATTTGCTATAATAAGAAAAATT  
CTTGTGACTTTAAAAAAA



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Figure 56

MLRAPGCLLRTSVAPAAALAAALLSSLARCSLLEPRDPVASSLSPYFGTKTRYEDVNPVLLSGFEAPWRDPELL  
EGTCTPVQLVALIRHGTRYPTVKQIRKLRQLHGLLQARGSRDGGASSTGSRDLGAALADWPLWYADWMDGQLVE  
KGRQDMRQLALRLASLFPALFSRENYGRLRLITSSKHRCMDSSAAFLQGLWQHYHFGLP?PDVADMEFGPPTVN  
DKLMRFFDHCEKFLTEVEKNATALYHVEAFKTGPEMQNILKKVAATLQVPVNDLNADLIQVAFFTCSEDLAIG  
VKSPWCDVFDIDDAKVLEYLNDLKQYWKRGYGYTINSRSSCTLFQDIFQHLDKAVEQKQRSQPISSPVILQFGH  
AETLLPLLSLMGYFKDKEPLTAYNYKKQMRKFRSGLIVPYASNLI FVLYHCENAKTPKEQFRVQMLLNEKVL?  
LAYSQETVSFYEDLKNHYKDILQSCQTSEECELARANSTSEDEL

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Figure 57

CGCGAGGCGCGGGGAGCCTGGGACCAGGAGCGAGAGCCGCTACCTGCAGCCGCGCCACGGCACGGCAGCCA  
CCATGGGCGCTCCTGCTGTGCTTCTGCTCCTGTGCGGAGTAGTGGATTTCCGCCAGAAGTTTGAGTATCACTACT  
CCTGAAGAGATGATTGAAAAGCCAAAGGGGAACTGCCTATCTGCCATGCAAATTTACGCTTAGTCCCGAAGA  
CCAGGGACCGCTGGACATCGAGTGGCTGATATCACCAGCTGATAATCAGAAGGTGGATCAAGTGATTATTTTAT  
ATTCTGGAGACAAAATTTATGATGACTACTATCCAGATCTGAAAGGCCGAGTACATTTTACGAGTAATGATCTC  
AAATCTGGTGATGCATCAATAAATGTAACGAATTTACAACCTGTGAGATATTGGCACATATCAGTGCAAAGTGAA  
AAAAGCTCCTGGTGTGCAAATAAGAAGATTCATCTGGTAGTTCTTGTAAAGCCTTCAGGTGCGAGATGTTACG  
TTGATGGATCTGAAGAAATTGGAAGTGACTTTAAGATAAAATGTGAACCAAAGAAGGTTCACTTCCATTACAG  
TATGAGTGGCAAAAATTTGTCTGACTCACAGAAAATGCCCACTTCATGGTTAGCAGAAATGACTTCATCTGTTAT  
ATCTGTAAAAAATGCCTCTTCTGAGTACTCTGGGACATACAGCTGTACAGTCAGAAACAGAGTGGGCTCTGATC  
AGTGCTCTGTTGCGTCTAAACGTTGTCCTCCTTCAAATAAAGCTGGACTAATTCAGGAGCCATTATAGGAAC  
TTGCTTGCTCTAGCGCTCATTTGGTCTTATCATCTTTTGCTGTGCTAAAAAGCGCAGAGAAGAAAAATAGAAAA  
GGAAGTTTCATCAGATATCAGGGAAGATGTGCCACCTCCAAAGAGCCGTACGTCCACTGCCAGAAGCTACATCG  
GCAGTAATCATTATCCCTGGGGTCCATGTCTCCTTCCAACATGGAAGGATATTCGAAGACTCAGTATAACCAA  
GTACCAAGTGAAGACTTTGAACGCACTCCTCAGAGTCCGACTCTCCACCTGCTAAGTTCAAGTACCCCTACAA  
GACTGATGGAATTACAGTTGTATAAATATGGACTACTGAAGAATCTGAAGTATGTATTATTTGACTTTATTTT  
AGGCCTCTAGTAAAGACTTAAATGTTTTTTAAAAAAGCACAAAGGCACAGAGATTAGAGGAGCTGTAAAGAACAC  
ATCTACTTTATGCAATGGCATTAGACATGTAAGTCAGATGTGTCATGTCAAAATTAGTACGAGCCAAATCTTTGT  
TAAAAAACCTATGTATAGTGACACTGATAGTTAAAGATGTTTTATTATATTTTCAATAACTACCACTAACAA  
ATTTTTAACTTTTCATATGCATATTCTGATATGTGGTCTTTTAGGAAAAGTATGGTTAATAGTTGATTTTTCAA  
AGGAAATTTTAAATTTCTACGTTCTGTTTAAATGTTTTTGCTATTTAGTTAAATACATTGAAGGAAAATACCCG  
TTCTTTTCCCCTTTTATGCACACAACAGAAACACGCGTTGTCATGCTCAAACCTATTTTTTATTTGCACTACA  
TGATTTTCACACAATTCTCTTAAACAACGACATAAAATAGATTTCTTGTATATAAATAACTTACATACGCTCCA  
TAAAGTAAATTTCAAAGGTGCTAGAACAAATCGTCCACTTCTACAGTGTCTCGTATCCAACAGAGTTGATGC  
ACAATATATAAATACTCAAGTCCAATATTAATAAATTTAGGCACTTGACTAATTTAATAAATTTCTCAAACCTA  
TATCAATATCTAAGTGATATATTTTTTAAGAAAGATTATTTCTCAATAACTTCTATAAAAAAAGTTTGATGG  
TTTGGCCCATCTAAGTCACTACTATTAGTAAGAATTTTAACTTTTAAATGTGTAGTAAGGTTTATTTCTACCTT  
TTTCTCAACATGACACCAACACAATCAAAAACGAAGTTAGTGAGGTGCTAACATGTGAGGATTATCCAGTGAT  
TCCGGTCACAATGCATTCCAGGAGGAGGTACCCATGTCACTGGAATTGGGCGATATGGTTTATTTTTCTTCCC  
TGATTTGGATAACCAAATGGAACAGGAGGAGGATAGTGATTCTGATGGCCATTCCTCGATACATTCTGGCTT  
TTTTCTGGGCAAAGGTTGCCACATTTGGAAGAGGTGGAATATAAGTTCTGAAATCTGTAGGGAAGAGAACACAT  
TAAGTTAATTTCAAAGGAAAAATCATCATCTATGTTCCAGATTTCTCATTAAAGACAAAGTTACCCACAACACT  
GAGATCAGATCTAAGTGACACTCCTATTGTGAGGTCTAAATACATTAAAAACCTCATGTGTAATAGGCGTATAA  
TGTATAACAGGTGACCAATGTTTTCTGAATGCATAAAGAAATGAATAAATCAACACAGTACTTCTTAAACAA  
CTTCAACCAAAAAAGACCAAAACATGGAACGAATGGAAGCTTGAAGGACATGCTTGTGTTTGTAGTCCAGTGGTTT  
CCACAGCTGGCTAAGCCAGGAGTCACTTTGAGGCTTTTAAATACAAAACATTGGAGCTGGAGGCCATTATCCTT  
AGCAAACCTAATGCAGAAACAGAAAATCAACTACCGCATGTTCTCACTTATAAGTGGGAGGTAATGATAAGAACT  
TATGAACACAAAGAAGGAAACAATAGACATTTGGAGTCTATTTGAGAGGGGAGGTTGGGAGAAGGAAAGGAGCA  
GAAAAGATAACTATTGAGTACTGCCTTCAACCTGGGTGATGAAATAATATGTACAACAAATCCCTGTGACACA  
TGTTTACCTATGGAACAAACCTTCATGTGTATCCCTAAACCTAAAATAAAAGTTAAAAAAGGAAAAAAGGAAAA  
AAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAA  
AAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAAAAGGAAAA

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Figure 58

MALLLCFVLLCGVVDFARSLSIITPEEMIEKAKGETAYLPCKFTLSPEDQGPLDIEWLISPADNQKVDQVILY  
SGDKIYDDYYPOLKGRVHFTSNDLKSGDASINVTNLQLSDIGTYQCKVKKAPGVANKKIHLVVLVKPSGACYV  
DGSEEIGSDFKIKCEPKESLPLQYEWQKLSOSQKMPTSWLAEMTSSVISVKNASSEYSGTYSCTVRNRVGSQQ  
CLLRNLNVPPSNKAGLIAGAIIGTLLALALIGLIIFCCRKKRREEKYEKEVHHDIREDVPPPKSRTSTARSYIG  
SNHSSLGSMSPSNMEGYSKTQYNQVPSSEDFERTPQSFTLPPAKFKYPYKTDGITVV

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Figure 59

CCTGGAGCCGGAAGCGGGCTGCAGCAGGGCGAGGCTCCAGGTGGGGTCGGTTCCGCATCCAGCCTAGCGTGTC  
CACGATGCGGCTGGGCTCCGGGACTTTCGCTACCTGTTGCGTAGCGATCGAGGTGCTAGGGATCGCGGTCTTCC  
TTCGGGGATTCTTCCCGGCTCCCGTTTCGTTCTCTGCCAGAGCGGAACACGGAGCGGAGCCCCAGCGCCGAA  
CCCTCGGCTGGAGCCAGTTCTAACTGGACCAGCTGCCACCACCTCTCTTCAGTAAAGTTGTTATTGTTCTGAT  
AGATGCCTTGAGAGATGATTTTGTGTTTGGGTCAAAGGGTGTGAAATTTATGCCCTACACAACCTACCTTGTGG  
AAAAAGGAGCATCTCACAGTTTGTGGCTGAAGCAAAGCCACCTACAGTTACTATGCCTCGAATCAAGGCATTG  
ATGACGGGGAGCCTTCTTGGCTTGTGCGACGTCATCAGGAACCTCAATTCTCTGCACTGCTGGAAGACAGTGT  
GATAAGACAAGCAAAAGCAGCTGGAAAAAGAATAGTCTTTTATGGAGATGAAACCTGGGTAAATATTCCCAA  
AGCATTTTGTGGAATATGATGGAACAACCTCATTTCGTTGTCAGATTACACAGAGGTGGATAAATGTCACG  
AGGCATTTGGATAAAGTATTAAGAGAGGAGATTGGGACATATTAATCCTCCACTACCTGGGGCTGGACCACAT  
TGGCCACATTTAGGGCCCAACAGCCCCCTGATTGGGCGAGAAGCTGAGCGAGATGGACAGCGTCTGATGAAGA  
TCCACACCTCACTGCAGTCGAAGGAGAGAGACGCCTTTACCCAATTGCTGGTTCTTTGTGGTGACCATGGC  
ATGTCGAAACAGGAAGTCACGGGGCCTCCTCCACCGAGGAGGTGAATACACCTCTGATTTTATCAGTTCTGC  
GTTTGAAAGGAAACCCGGTGATATCCGACATCCAAAGCACGTCCAATAGACGGATGTGGCTGCGACACTGGCGA  
TAGCACTTGGCTTACCGATTCCAAAAGACAGTGTAGGGAGCCTCCTATTCCAGTTGTGGAAGGAAGACCAATG  
AGAGAGCAGTTGAGATTTTACATTTGAATACAGTGCAGCTTAGTAACTGTTGCAAGAGAATGTGCCGTGCTA  
TGAAAAGATCCTGGGTTTGAGCAGTTTAAATGTGAGAAAGATTGCATGGGAAGTGGATCAGACTGTACTTGG  
AGGAAAAGCATTGAGAGTCTTCAACCTGGGCTCCAAGGTTCTCAGGCAGTACCTGGATGCTCTGAAGACG  
CTGAGCTTGTCCCTGAGTGCACAAGTGGCCCACTTCTCACCTGCTCCTGCTCAGCGTCCCACAGGCAGTGCAC  
AGAAAGGCTGAGCTGGAAGTCCCACTGTCTCTCTGGGTTTCTCTGCTCTTTTATTTGGTGATCCTGGTTCT  
TTCGGCCGTTACGTCATTGTGTGCACCTCAGCTGAAAGTTCGTGCTACTTCTGTGGCCTCTCGTGGCTGGCGG  
CAGGCTGCCTTTTCGTTTACCAGACTCTGGTTGAACACCTGGTGTGTGCCAAGTGTGGCAGTGCCTGGACAGG  
GGGCCTCAGGGAAGGACGTGGAGCAGCCTTATCCAGGCCCTCTGGGTGTCCCGACACAGGTGTTACATCTGTG  
CTGTGAGGTCAGATGCCTCAGTTCTTGAAAGCTAGGTTCTGCGACTGTTACCAAGGTGATTGTAAAGAGCTG  
GCGGTACAGAGGAACAAGCCCCCAGCTGAGGGGGTGTGTGAATCGGACAGCCTCCAGCAGAGGTGTGGGAG  
CTGCAGCTGAGGGAAGAAGAGACAATCGGCCTGGACACTCAGGAGGGTCAAAGGAGACTTGGTGCACCACTC  
ATCCTGCCACCCCAAGATGCATCCTGCCTCATCAGGTCCAGATTTCTTTCCAAGGCGGACGTTTCTGTTGGA  
ATTCTTAGTCCTTGGCCTCGGACACCTTCATTGTTAGCTGGGGAGTGGTGGTGAGGCAGTGAAGAAGAGGCGG  
ATGGTCACACTCAGATCCACAGAGCCAGGATCAAGGGACCCACTGCAGTGGCAGCAGGACTGTTGGGCCCCCA  
CCCCAACCTGCACAGCCCTCATCCCCCTTGGCTTGAGCCGTGAGAGGCCCTGTGCTGAGTGTCTGACCGAGA  
CACTCACAGCTTTGTATCAGGGCACAGGCTTCTCGGAGCCAGGATGATCTGTGCCACGCTTGACCTCGGGC  
CCATCTGGGCTCATGCTCTCTCTCTGCTATTGAATTAGTACCTAGCTGCACACAGTATGTAGTTACCAAAAGA  
ATAAACGGCAATAATTGAGAAAAAAA

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Figure 60

MRLGSGTFATCCVAIEVLGIAVFLRGFFPAPVRSSARAEHGAEPPEPSAGASSNWTTLPPPLFSKVIVLID  
ALRDDFVFGSKGVKEMPYTTYLVEKGASHSEVAEAKPPTVTMPRIKALMTGSLPGFVDVIRNLNSPALLEDSEVI  
RQAKAAGKRIVFYGDETWVKLFPHKFVEYDGTTSFFVSDYTEVDNNVTRHLDKVLKRGDWDILHLYLGLOHIG  
HISGPNNSPLIGQKLSEMDSVLMKIHTSLQSKERETPLPNLLVLCGDHGMSETGSHGASSTEEVNTPLILISSAF  
ERKPGDIRHPKHVQ

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Figure 61

GGGTGATTGAACTAAACCTTCGCCGCACCGAGTTTGCAGTACGGCCGTACCCGCACCGCTGCCTGCTTGCGGT  
TGGAGAAATCAAGGCCCTACCGGGCCTCCGTAGTCACCTCTCTATAGTGGGCGTGGCCGAGGCCGGGGTGACCC  
TGCCGGAGCCTCCGCTGCCAGCGACATGTTCAAGGTAATTCAGAGGTCCGTGGGGCCAGCCAGCCTGAGCTTGC  
TCACCTTCAAAGTCTATGCAGCACCAAAAAGGACTCACCTCCAAAAATTCCTGTAAGGTTGATGAGCTTTCA  
CTCTACTCAGTTCTGAGGGTCAATCGAAGTATGTGGAGGAGGCAAGGAGCCAGCTTGAAGAAAGCATCTCACA  
GCTCCGACACTATTGCGAGCCATACACAACCTGGTGTCAAGGAAACGTACTCCAAACTAAGCCCAAGATGCAAA  
GTTTGGTTCAATGGGGGTTAGACAGCTATGACTATCTCCAAATGCACCTCCTGGATTTTTTCCGAGACTTGGT  
GTTATTGGTTTTGCTGGCCTTATTGGACTCCTTTTGGCTAGAGGTTCAAAAATAAAGAAGCTAGTGTATCCGCC  
TGGTTTCATGGGATTAGCTGCCTCCCTCTATTATCCACAACAAGCCATCGTGTGTTGCCAGGTGAGTGGGGAGA  
GATTATATGACTGGGGTTTACGAGGATATATAGTCATAGAAGATTGTGGAAGGAGAACTTTCAAAGCCAGGA  
AATGTGAAGAATTACCTGGAACCTAAGTAGAAACTCCATGCTCTGCCATCTTAATCAGTTATAGGTAAACATT  
GGAAACTCCATAGAATAAATCAGTATTTCTACAGAAAAATGGCATAGAAGTCAGTATTGAATGTATTAATTTGG  
CTTTCTTCTTCAGGAAAACTAGACCAGACCTCTGTTATCTTCTGTGAAATCATCCTACAAGCAAACTAACCTG  
GAATCCCTTCACCTAGAGATAATGTACAAGCCTTAGAACTCCTCATTCTCATGTTGCTATTTATGTACCTAATT  
AAAACCCAAGTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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Figure 62

MEKVIQRSVGFASLSLLTFKVYAAPKKDSPPKNSVKVDELSLYSVPEGQSKYVEEARSQLEESISQLRHYCEPY  
TTWCQETYSQTKPKMQSLVQWGLDSYDYLQNAFFGFFPRLGVIGFAGLIGLLLARGSKIKKLVYPPGFMGLAAS  
LYYPQQAIVFAQVSGERLYDWGLRGYIVIEDLWKENFQKPGNVKNSPGTK

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Figure 63

AAGGAGCAGCCCCGAAGCACCAAGTGAGAGGCATGAAGTTACAGTGTGTTTCCCTTTGGCTCCTGGGTACAATA  
CTGATATTGTGCTCAGTAGACAACCACGGTCTCAGGAGATGTCTGATTTCCACAGACATGCACCATATAGAAGA  
GAGTTTCCAAGAAATCAAAAGAGCCATCCAAGCTAAGGACACCTTCCCAAATGTCACTATCCTGTCCACATTGG  
AGACTCTGCAGATCATTAGCCCTTAGATGTGTGCTGCGTGACCAAGAACCTCCTGGCGTTCTACGTGGACAGG  
GTGTTCAAGGATCATCAGGAGCCAAACCCCAAAATCTTGAGAAAAATCAGCAGCATTGCCAACTCTTTCCTCTA  
CATGCAGAAAACCTCTGCGGCAATGTCAGGAACAGAGGCAGTGTCACTGCAGGCAGGAAGCCACCAATGCCACCA  
GAGTCATCCATGACAACTATGATCAGCTGGAGGTCCACGCTGCTGCCATTAAATCCCTGGGAGAGCTCGACGTC  
TTTCTAGCCTGGATTAATAAGAATCATGAAGTAATGTTCTCAGCTTGATGACAAGGAACCTGTATAGTGATCCA  
GGGATGAACACCCCTGTGCGGTTTACTGTGGGAGACAGCCACCTTGAAGGGGAAGGAGATGGGGAAGGCCCC  
TTGCAGCTGAAAGTCCCCTGGCTGGCCTCAGGCTGTCTTATTCCGCTTGAAAATAGGCCAAAAGTCTACTGTG  
GTATTTGTAATAAACTCTATCTGCTGAAAGGGCCTGCAGGCCATCCTGGGAGTAAAGGGCTGCCTTCCCATCTA  
ATTTATTGTAAAGTCATATAGTCCATGTCTGTGATGTGAGCCAAGTGATATCCTGTAGTACACATTGTACTGAG  
TGGTTTTTCTGAATAAATTCCATATTTTACCTATGA



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Figure 64

MKLQCVSLWLLGTILILCSVDNHGLRRCLISTDMHHIEESFQEIKRAIQAKDTFPNVTILSTLETLQIIKPLDV  
CCVTKNLLAFYVDRVFKDHQEPNPKILRKISSIANSFLYMQKTLRQCQEQRQCHCRQEATNATRVIHNDNYDQLE  
VHAAAIKSLGELDVFLAWINKNHEVMESA

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Figure 65

[illegible]

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Figure 66

MAPARAGFCPLLLLLLLGLWVAEIPVSAKPKGMTSSQWFKIQHMQPSPQACNSAMKNINKHTKRCCKDLNTFLHE  
PTSSVAATCQTPKIAKNGDKNCHQSHGPFVSLTMCKLTSGKYPNCRYKEKRONKSYVVACKPPQKKDSQQFHLV  
PVHLDRV L

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P2933R1

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0-1	Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared using	PCT-EASY Version 2.90 (updated 10.05.2000)
0-2	International Application No.	
0-3	Applicant's or agent's file reference	P2933R1
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	123
1-2	line	7
1-3	Identification of Deposit	
1-3-1	Name of depositary institution	American Type Culture Collection
1-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
1-3-3	Date of deposit	16 September 1997 (16.09.1997)
1-3-4	Accession Number	ATCC 209254
1-4	Additional Indications	NONE
1-5	Designated States for Which Indications are Made	all designated States
1-6	Separate Furnishing of Indications	NONE
	These indications will be submitted to the International Bureau later	
2	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
2-1	page	123
2-2	line	8
2-3	Identification of Deposit	
2-3-1	Name of depositary institution	American Type Culture Collection
2-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
2-3-3	Date of deposit	16 September 1997 (16.09.1997)
2-3-4	Accession Number	ATCC 209265
2-4	Additional Indications	NONE
2-5	Designated States for Which Indications are Made	all designated States
2-6	Separate Furnishing of Indications	NONE
	These indications will be submitted to the International Bureau later	
3	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
3-1	page	123
3-2	line	9

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3-3	Identification of Deposit	
3-3-1	Name of depositary institution	American Type Culture Collection
3-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
3-3-3	Date of deposit	17 October 1997 (17.10.1997)
3-3-4	Accession Number	ATCC 209401
3-4	Additional Indications	NONE
3-5	Designated States for Which Indications are Made	all designated States
3-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
4	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
4-1	page	123
4-2	line	10
4-3	Identification of Deposit	
4-3-1	Name of depositary institution	American Type Culture Collection
4-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
4-3-3	Date of deposit	07 November 1997 (07.11.1997)
4-3-4	Accession Number	ATCC 209435
4-4	Additional Indications	NONE
4-5	Designated States for Which Indications are Made	all designated States
4-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
5	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
5-1	page	123
5-2	line	11
5-3	Identification of Deposit	
5-3-1	Name of depositary institution	American Type Culture Collection
5-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
5-3-3	Date of deposit	
5-3-4	Accession Number	ATCC unknown
5-4	Additional Indications	NONE
5-5	Designated States for Which Indications are Made	all designated States
5-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE

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6	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
6-1	page	123
6-2	line	12
6-3	Identification of Deposit	
6-3-1	Name of depositary institution	American Type Culture Collection
6-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
6-3-3	Date of deposit	26 March 1998 (26.03.1998)
6-3-4	Accession Number	ATCC 209704
6-4	Additional Indications	NONE
6-5	Designated States for Which Indications are Made	all designated States
6-6	Separate Furnishing of Indications	NONE
These indications will be submitted to the International Bureau later		
7	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
7-1	page	123
7-2	line	13
7-3	Identification of Deposit	
7-3-1	Name of depositary institution	American Type Culture Collection
7-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
7-3-3	Date of deposit	28 April 1998 (28.04.1998)
7-3-4	Accession Number	ATCC 209808
7-4	Additional Indications	NONE
7-5	Designated States for Which Indications are Made	all designated States
7-6	Separate Furnishing of Indications	NONE
These indications will be submitted to the International Bureau later		
8	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
8-1	page	132
8-2	line	14
8-3	Identification of Deposit	
8-3-1	Name of depositary institution	American Type Culture Collection
8-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
8-3-3	Date of deposit	07 November 1997 (07.11.1997)
8-3-4	Accession Number	ATCC 209436
8-4	Additional Indications	NONE
8-5	Designated States for Which Indications are Made	all designated States

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8-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
9	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
9-1	page	123
9-2	line	15
9-3	Identification of Deposit	
9-3-1	Name of depositary institution	American Type Culture Collection
9-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
9-3-3	Date of deposit	28 November 1997 (28.11.1997)
9-3-4	Accession Number	ATCC 209422
9-4	Additional Indications	NONE
9-5	Designated States for Which Indications are Made	all designated States
9-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
10	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
10-1	page	123
10-2	line	16
10-3	Identification of Deposit	
10-3-1	Name of depositary institution	American Type Culture Collection
10-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
10-3-3	Date of deposit	26 March 1998 (26.03.1998)
10-3-4	Accession Number	ATCC 209705
10-4	Additional Indications	NONE
10-5	Designated States for Which Indications are Made	all designated States
10-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
11	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
11-1	page	123
11-2	line	17
11-3	Identification of Deposit	
11-3-1	Name of depositary institution	American Type Culture Collection
11-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
11-3-3	Date of deposit	23 April 1998 (23.04.1998)
11-3-4	Accession Number	ATCC 209804

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11-4	Additional Indications	NONE
11-5	Designated States for Which Indications are Made	all designated States
11-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
12	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
12-1	page	123
12-2	line	18
12-3	Identification of Deposit	
12-3-1	Name of depositary institution	American Type Culture Collection
12-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
12-3-3	Date of deposit	06 May 1998 (06.05.1998)
12-3-4	Accession Number	ATCC 209849
12-4	Additional Indications	NONE
12-5	Designated States for Which Indications are Made	all designated States
12-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
13	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
13-1	page	123
13-2	line	19
13-3	Identification of Deposit	
13-3-1	Name of depositary institution	American Type Culture Collection
13-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
13-3-3	Date of deposit	23 June 1998 (23.06.1998)
13-3-4	Accession Number	ATCC 203017
13-4	Additional Indications	NONE
13-5	Designated States for Which Indications are Made	all designated States
13-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
14	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
14-1	page	123
14-2	line	20



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14-3	Identification of Deposit	
14-3-1	Name of depositary institution	American Type Culture Collection
14-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
14-3-3	Date of deposit	12 January 1999 (12.01.1999)
14-3-4	Accession Number	ATCC 203583
14-4	Additional Indications	NONE
14-5	Designated States for Which Indications are Made	all designated States
14-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
15	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
15-1	page	123
15-2	line	21
15-3	Identification of Deposit	
15-3-1	Name of depositary institution	American Type Culture Collection
15-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
15-3-3	Date of deposit	05 July 1996 (05.07.1996)
15-3-4	Accession Number	ATCC 55820
15-4	Additional Indications	NONE
15-5	Designated States for Which Indications are Made	all designated States
15-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
16	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
16-1	page	123
16-2	line	22
16-3	Identification of Deposit	
16-3-1	Name of depositary institution	American Type Culture Collection
16-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
16-3-3	Date of deposit	01 July 1998 (01.07.1998)
16-3-4	Accession Number	ATCC 203046
16-4	Additional Indications	NONE
16-5	Designated States for Which Indications are Made	all designated States
16-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE

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17	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
17-1	page	123
17-2	line	23
17-3	Identification of Deposit	
17-3-1	Name of depositary institution	American Type Culture Collection
17-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
17-3-3	Date of deposit	14 May 1998 (14.05.1998)
17-3-4	Accession Number	ATCC 209866
17-4	Additional Indications	NONE
17-5	Designated States for Which Indications are Made	all designated States
17-6	Separate Furnishing of Indications	NONE
	These indications will be submitted to the International Bureau later	
18	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
18-1	page	123
18-2	line	24
18-3	Identification of Deposit	
18-3-1	Name of depositary institution	American Type Culture Collection
18-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
18-3-3	Date of deposit	15 December 1998 (15.12.1998)
18-3-4	Accession Number	ATCC 203540
18-4	Additional Indications	NONE
18-5	Designated States for Which Indications are Made	all designated States
18-6	Separate Furnishing of Indications	NONE
	These indications will be submitted to the International Bureau later	
19	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
19-1	page	123
19-2	line	25
19-3	Identification of Deposit	
19-3-1	Name of depositary institution	American Type Culture Collection
19-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
19-3-3	Date of deposit	04 August 1998 (04.08.1998)
19-3-4	Accession Number	ATCC 203092
19-4	Additional Indications	NONE
19-5	Designated States for Which Indications are Made	all designated States

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19-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
20	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
20-1	page	123
20-2	line	26
20-3	Identification of Deposit	
20-3-1	Name of depositary institution	American Type Culture Collection
20-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
20-3-3	Date of deposit	17 November 1998 (17.11.1998)
20-3-4	Accession Number	ATCC 203464
20-4	Additional Indications	NONE
20-5	Designated States for Which Indications are Made	all designated States
20-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
21	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
21-1	page	123
21-2	line	27
21-3	Identification of Deposit	
21-3-1	Name of depositary institution	American Type Culture Collection
21-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
21-3-3	Date of deposit	15 September 1998 (15.09.1998)
21-3-4	Accession Number	ATCC 203235
21-4	Additional Indications	NONE
21-5	Designated States for Which Indications are Made	all designated States
21-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
22	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
22-1	page	123
22-2	line	28
22-3	Identification of Deposit	
22-3-1	Name of depositary institution	American Type Culture Collection
22-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
22-3-3	Date of deposit	22 September 1998 (22.09.1998)
22-3-4	Accession Number	ATCC 203282

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22-4	Additional Indications	NONE
22-5	Designated States for Which Indications are Made	all designated States
22-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
23	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
23-1	page	123
23-2	line	29
23-3	Identification of Deposit	
23-3-1	Name of depositary institution	American Type Culture Collection
23-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
23-3-3	Date of deposit	11 August 1998 (11.08.1998)
23-3-4	Accession Number	ATCC 203115
23-4	Additional Indications	NONE
23-5	Designated States for Which Indications are Made	all designated States
23-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
24	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
24-1	page	123
24-2	line	30
24-3	Identification of Deposit	
24-3-1	Name of depositary institution	American Type Culture Collection
24-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
24-3-3	Date of deposit	22 September 1998 (22.09.1998)
24-3-4	Accession Number	ATCC 203276
24-4	Additional Indications	NONE
24-5	Designated States for Which Indications are Made	all designated States
24-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
25	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
25-1	page	123
25-2	line	31

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25-3	Identification of Deposit	
25-3-1	Name of depositary institution	American Type Culture Collection
25-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
25-3-3	Date of deposit	22 September 1998 (22.09.1998)
25-3-4	Accession Number	ATCC 203270
25-4	Additional Indications	NONE
25-5	Designated States for Which Indications are Made	all designated States
25-6	Separate Furnishing of Indications	NONE
	These indications will be submitted to the International Bureau later	
26	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
26-1	page	123
26-2	line	32
26-3	Identification of Deposit	
26-3-1	Name of depositary institution	American Type Culture Collection
26-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
26-3-3	Date of deposit	12 January 1999 (12.01.1999)
26-3-4	Accession Number	ATCC 203573
26-4	Additional Indications	NONE
26-5	Designated States for Which Indications are Made	all designated States
26-6	Separate Furnishing of Indications	NONE
	These indications will be submitted to the International Bureau later	
27	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
27-1	page	123
27-2	line	33
27-3	Identification of Deposit	
27-3-1	Name of depositary institution	American Type Culture Collection
27-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209United States of America
27-3-3	Date of deposit	27 April 1999 (27.04.1999)
27-3-4	Accession Number	ATCC 203966
27-4	Additional Indications	NONE
27-5	Designated States for Which Indications are Made	all designated States
27-6	Separate Furnishing of Indications	NONE
	These indications will be submitted to the International Bureau later	

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28	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
28-1	page	123
28-2	line	34
28-3	Identification of Deposit	
28-3-1	Name of depositary institution	American Type Culture Collection
28-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
28-3-3	Date of deposit	09 March 1999 (09.03.1999)
28-3-4	Accession Number	ATCC 203834
28-4	Additional Indications	NONE
28-5	Designated States for Which Indications are Made	all designated States
28-6	Separate Furnishing of Indications	NONE
	These indications will be submitted to the International Bureau later	
29	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
29-1	page	123
29-2	line	35
29-3	Identification of Deposit	
29-3-1	Name of depositary institution	American Type Culture Collection
29-3-2	Address of depositary institution	10801 University Blvd., Manassas, Virginia 20110-2209 United States of America
29-3-3	Date of deposit	20 July 1999 (20.07.1999)
29-3-4	Accession Number	ATCC PTA-387
29-4	Additional Indications	NONE
29-5	Designated States for Which Indications are Made	all designated States
29-6	Separate Furnishing of Indications	NONE
	These indications will be submitted to the International Bureau later	

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0-4	This form was received with the international application: (yes or no)	
0-4-1	Authorized officer	

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0-5	This form was received by the international Bureau on:	
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0-5-1	Authorized officer	
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